REMARKS/ARGUMENTS

At this time, Applicants wish to thank the Examiner for his time and comments during the telephone interview of November 19, 2002. During the telephone interview, all the prior art references were discussed. In particular, Applicants stressed that the prior art references taught the use of lactoferrin to treat bacterial and/or viral infections, which is not similar to the present invention which uses lactoferrin to treat an allergen-induced inflammatory response. Thus, it was agreed that Applicants would supply definitions of allergen and describe the differences between the immune response to a bacterial and/or viral infection versus an allergen induced immune response.

I. Definitions

At this time, Applicants provide definitions for the terms allergen, allergic reaction, hypersensitivity reactions and infection. These terms are well known and understood by those of skill in the art.

> Allergens are defined as antigens that elicit hypersensitivity or allergic reactions. (ImmunoBiology 4th Ed., page 593, 1999).
>
> An allergic reaction is a response to innocuous environmental antigens or allergens due to pre-existing antibody or T cells. (ImmunoBiology 4th Ed., page 594, 1999).
>
> Hypersensitivity reactions are immune responses to innocuous antigens that lead to symptomatic reactions upon re-exposure. (ImmunoBiology 4th Ed., page 602, 1999).
>
> Infection is invasion by and multiplication of pathogenic microorganisms in a body tissue. (American Heritage College Dictionary 3rd Ed., page 696, 1997.

The present invention is drawn to the use of lactoferrin to prevent or treat an allergen-induced inflammatory response. The mechanisms involved in an allergen-induced immune response differs from an immune response resulting from an insult by a pathogenic agent, such as bacteria or viruses. Inflammation that occurs via a pathogenic agent is typically a result of endotoxin toxicity. Endotoxins are toxins that are released from the pathogen. Thus, an endotoxin is not innocuous; it is toxic to the cell and triggers phagocytes to release cytokines that produce local or systemic symptoms. An allergen-induced inflammatory response results from the immune system responding to an innocuous agent. For the

convenience of the Examiner, Applicants are including herewith copies of two book chapters that provides further explanation of the immune system in response to pathogens and the immune system and its role in hypersensitivity reactions. (ImmunoBiology 4th Ed., 1999, Chapter 10 and Chapter 12).

II. Status of Application

Claims 5-10, 12-14, and 21-25 are pending in this application. Claim 21 has been amended, claims 12-14, 22 and 25 have been canceled, and claims 26-29 have been added to clarify the scope of the present invention. Applicants attach as Appendix A, a version of the claims showing amendments herein. Also, for the convenience of the Examiner, Applicants have attached as Appendix B a version of the claims pending as of this amendment. No new matter has been added.

The issues outstanding in this application are as follows:

- Claims 5-10, 12-14 and 21-25 were rejected under 35 U.S.C. §103(a), which the Examiner alleges that the claimed subject matter is unpatentable over Teng et al. in view of Britigan, Greff and De Lacharriere et al.

- Claims 5-10, 12-14 and 21-25 were rejected under 35 U.S.C. §103(a), which the Examiner alleges that the claimed subject matter is unpatentable over Teng et al. in view of Nuijens et al., Enk et al., Database WPI AN 95-340208 and Penco et al.

Applicants respectfully traverse the outstanding rejections and objections, and applicants respectfully request reconsideration and withdrawal thereof in light of the amendments and remarks contained herein.

III. Teng *et al.* in combination of Britigan *et al.*, Greff, and De Lacharriere *et al.*

Claims 5-10, 12-14 and 21-25 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over the primary reference Teng *et al.* in combination of the above-listed references. The Examiner states that Teng *et al.* teaches a method of treating human dermal inflammatory disorder by administering a pharmaceutically effective amount of lactoferrin product, citing the disclosure at page 4, lines 21-30 of Teng *et al.*

The MPEP sets forth the guidelines to establish a *prima facie* case of obviousness under 35 U.S.C. § 103 (MPEP § 2143.3). Three basic criteria must be met to establish a *prima facie* case of obviousness. The three criteria are:

> 1) a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
>
> 2) a reasonable expectation of success; and
>
> 3) the prior art references must teach or suggest all the claim limitations.

In view of the above criteria, Applicants assert that the Office has not established a *prima facie* case of obviousness to reject the claims under 35 U.S.C. § 103 in light of the above criteria. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, (Fed. Cir. 1991). A *prima facie* case necessitates disclosure of the source for either a suggestion or motivation to modify a reference to produce the present invention, and a reasonable expectation of success of producing the present invention. A *prima facie* case must be established by evidence rather than conjecture. *Ex parte Yamamoto*, 57 USPQ2d 1382, 1383, 1384 (CCPA 2000). In the present case, it is mere conjecture on the part of the Office that one of skill in the art would be able use the lactoferrin composition in Teng et al. in combination with the lactoferrin compositions described in Britigan et al., Greff et al., and the TNF α antagonists described in De Lacharriere to develop the lactoferrin composition of the present invention to treat an allergen-induced inflammatory response.

In light of the discussion under the definition section, Applicants assert that Teng et al., Britigan et al., and Greff do not teach or suggest the use of lactoferrin to treat an allergen-induced inflammatory response. The present application, on page 3, lines 20-21, indicates that lactoferrin inhibits allergen-induced inflammation that is not induced by an endotoxin, such as lipopolysaccharide (LPS). Teng et al. teaches the use of lactoferrin as a treatment for antibacterial and antiviral infections (see page 4, lines 21-30 and page 13, lines 1-5). Britigan et al. also teaches the use of lactoferrin to treat bacterial infections via scavening free radicals that are produced by phagocytosis. In fact, Britigan et al. further suggests that lactoferrin may play a role in ameliorating LPS-induced toxicity (see page 151, last sentence of summary). Greff also teaches the use of lactoferrin to scavenge free radicals. Thus,

Applicants assert that the combination of Teng et al., Britigan et al. and Greff teach the antimicrobial activity of lactoferrin (lactoferrin's activity against a pathogen), however, the references do not teach or suggest the use of lactoferrin to treat an allergen-induced immune response (lactoferrin's activity against an innocuous agent).

De Lacharriere is cited for teaching the use of TNF α antagonists in pharmaceutical compositions. De Lacharriere does not describe that lactoferrin is a TNF antagonist. In fact, the term "TNF α antagonists" is defined purely in functional terms, see column 3 lines 4-10 of the De Lacharriere patent. It is stated that "*all substances capable of inhibiting the release and/or synthesis and/or receptor binding of ...TNF alpha*" are considered "TNF α antagonists." Without teaching the structures of potential TNF α antagonists, no one in the art would know how to select a candidate from millions of natural and recombinant biological molecules in order to test for its ability to inhibit TNF α production. Such a general statement in De Lacharriere cannot be fairly construed as providing a suggestion for one skilled in the art to select lactoferrin, and specifically, to test its ability to inhibit TNF α production in dermal cells, and to conduct the test under the particular condition that the mammal has been inflicted with an allergen.

Applicants contend that the teachings of Teng et al., Britigan et al., Greff and De Lacharriere alone or in combination do not teach nor suggest using lactoferrin to treat an allergen-induced inflammatory response. These references teach the use of lactoferrin to inhibit LPS toxicity, which LPS toxicity is not involved in an allergen-induced response. Thus, with the lack of teaching or suggestion, Applicants assert that the references do not meet the basic requirements of a *prima facie* case of obvious and respectfully request that the rejection be withdrawn.

IV. Teng *et al.* in combination of Nuijens *et al.*, and Enk et al, Database WPI AN 95-340208, and Penco et al

Claims 5-10, 12-14 and 21-25 stand rejected under 35 U.S.C. § 103 (a) as allegedly being unpatentable over the primary reference Teng *et al.* in combination of the above-listed references, all of which are of record.

The MPEP sets forth the guidelines to establish a *prima facie* case of obviousness under 35 U.S.C. § 103 (MPEP § 2143.3). Three basic criteria must be met to establish a

prima facie case of obviousness. The three criteria are:

> 1) a suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
>
> 2) a reasonable expectation of success; and
>
> 3) the prior art references must teach or suggest all the claim limitations.

As explained in the section above, the primary reference, Teng et al., fails to teach or suggest the use of lactoferrin to treat an allergen-induced inflammatory response. Applicants also contend that the secondary references do not teach or suggest that lactoferrin can be used to treat allergen-induced inflammatory responses.

Nuijens *et al.* reports that lactoferrin suppresses IL-1 and TNF-α release from monocytes in response to LPS from Gram-negative bacteria. See last paragraph at page 287 that is cited by the Examiner. Nuijens does not teach or suggest that lactoferrin suppresses IL-1 or TNF-α production from dermal cells in response to an allergen, which is mediated through an LPS independent pathway. As such, Nuijens *et al.* is not on point and adds nothing to the notion of using lactoferrin for treatment of dermal inflammation, and more specifically allergen-induced inflammation.

Similarly, AN 95-340208 teaches the preparation and use of a lactoferrin composition that confers antimicrobial activity, which again relates to LPS pathway as stated and distinguished in the specification. As such, AN 95-340208 fails to appreciate the anti-allergen activity of lactoferrin and fails to describe other required elements of the invention.

Enk *et al.* and Penco et al. report *in vitro* studies on two cytokines, namely IL-1β and TNF-α. Neither reference fairly suggests the relevance, if any, of the *in vitro* test to an *in vitro* application of lactoferrin.

In light of the above arguments, Applicants assert that the Office has not established a *prima facie* case of obviousness to reject the claims under 35 U.S.C. § 103. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, (Fed. Cir. 1991). A *prima facie* case necessitates disclosure of the source for either a suggestion or motivation to modify a reference to produce the present

invention, and a reasonable expectation of success of producing the present invention. A *prima facie* case must be established by evidence rather than conjecture. *Ex parte Yamamoto*, 57 USPQ2d 1382, 1383, 1384 (CCPA 2000). In the present case, it is mere conjecture on the part of the Office that one of skill in the art would be able use the lactoferrin composition in Teng et al. in combination with the lactoferrin compositions described in Nuijens et al. and AN95-340208 and the cytokine teachings of Enk et al., and Penco et al. to develop the lactoferrin composition of the present invention to treat an allergen-induced inflammatory response. Applicants contend that the teachings of Teng et al., Nuijens et al., AN95-340208, Enk et al., and Penco et al. do not teach nor suggest using lactoferrin to treat an allergen induced inflammatory response. These references teach the use of lactoferrin to inhibit LPS toxicity, which LPS toxicity is not involved in an allergen induced response. Thus, with the lack of teaching or suggestion, Applicants assert that the references do not meet the basic requirements of a *prima facie* case of obvious.

Thus, with the lack of teaching or suggestion, Applicants assert that the references do not meet the basic requirements of a *prima facie* case of obvious and respectfully request that the rejection be withdrawn.

CONCLUSION

Applicants have amended claim 21 without prejudice and without acquiescence to clarify the present claimed invention. Claims 12-14, 22 and 25 have been canceled without prejudice and without acquiescence. And claims 26-29 have been added. Applicants believe that there are no fees associated with the filing of this document. However, the Commissioner is hereby authorized to any required fees associated with this filing, to Deposit Account No. 06-2375, under Order No. 10211629, from which the undersigned is authorized to draw.

Applicants assert that in view of the above remarks, that the application is now considered for allowance. Accordingly, Applicants request that a Letter Patent be issued on the application as herein amended. If any requirements remain, please contact the undersigned for quick resolution.

Dated: February 10, 2003

Respectfully submitted,

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Appendix A
Showing amendments made herein

21. (Amended Twice) A method of inhibiting in a mammal a dermal allergen-induced inflammatory response comprising administering to the mammal a therapeutically effective amount of lactoferrin product, said amount being sufficient to reduce inflammation-induced Langerhans cell migration or accumulation of dendritic cells in lymph nodes.

Appendix B
Pending claims as the date of this Amendment

5. A method of treating an allergen-induced inflammatory disorder in a mammal, comprising the step of administering to a mammal a therapeutically effective amount of a lactoferrin product, said amount being sufficient to reduce inflammation-induced Langerhans cell migration or accumulation of dendritic cells in lymph nodes.

6. The method of claim 5, wherein the mammal is a human.

7. The method according to claim 5 wherein the allergen-induced inflammatory disorder features a local immune response characterized by increased production of TNF-α.

8. The method according to claim 5 wherein the lactoferrin product is a naturally occurring lactoferrin.

9. The method according to claim 5 wherein the lactoferrin product is a recombinantly produced lactoferrin or a biologically active analog thereof.

10. The method according to claim 5 wherein the lactoferrin product is a biologically active fragment of lactoferrin.

21. A method of inhibiting in a mammal a dermal allergen-induced inflammatory response comprising administering to the mammal a therapeutically effective amount of lactoferrin product, said amount being sufficient to reduce inflammation-induced Langerhans cell migration or accumulation of dendritic cells in lymph nodes.

23. The method of claim 21, wherein the dermal inflammatory response is mediated by TNF-α.

24. The method of claim 21, wherein the dermal inflammatory response is further characterized by Langerhans cell migration.

26. The method of claim 5, wherein the allergen-induced inflammatory disorder is contact dermatitis.

27. The method of claim 21, wherein the dermal allergen-induced inflammatory response is manifested as a contact dermatitis.

28. The method of claim 5, wherein the allergen-induced inflammatory disorder is psoriasis.

29. The method of claim 21, wherein the dermal allergen-induced inflammatory response is manifested as psoriasis.

GLOSSARY

The **12/23** rule states that gene segments of immunoglobulin or T-cell receptors can be joined only if one has a recognition signal sequence with a 12 base pair spacer, and the other has a 23 base pair spacer.

In the context of immunoglobulins, α is the type of heavy chain in IgA.

The **ABO blood group system** antigens are expressed on red blood cells. They are used for typing human blood for transfusion. If they do not express A or B antigens on their red blood cells, people naturally form antibodies that interact with them.

The removal of antibodies specific for one antigen from an antiserum to render it specific for another antigen or antigens is called **absorption**.

α:β T-cell receptor: see **T-cell receptor**.

Accessory cells in adaptive immunity are cells that aid in the response but do not directly mediate specific antigen recognition. They include phagocytes, mast cells, and NK cells, and are also known as **accessory effector cells**.

The **acquired immune deficiency syndrome (AIDS)** is a disease caused by infection with the human immunodeficiency virus (HIV). AIDS occurs when an infected patient has lost most of his or her CD4 T cells, so that infections with opportunistic pathogens occur.

Acquired immune response: see **adaptive immune response**.

Immunization with antigen is called **active immunization** to distinguish it from the transfer of antibody to an unimmunized individual, which is called passive immunization.

Acute lymphoblastic leukemia is a highly aggressive, undifferentiated form of lymphoid malignancy derived from a progenitor cell that is thought to be able to give rise to both lineages of lymphoid cells.

Acute-phase proteins are a series of proteins found in the blood shortly after the onset of an infection. These proteins participate in early phases of host defense against infection. An example is the mannose-binding protein.

The **acute-phase response** is a change in the blood that occurs during early phases of an infection. It includes the production of acute-phase proteins and also of cellular elements.

The **adaptive immune response** or **adaptive immunity** is the response of antigen-specific lymphocytes to antigen, including the development of immunological memory. Adaptive immune responses are generated by clonal selection of lymphocytes. Adaptive immune responses are distinct from innate and non-adaptive phases of immunity, which are not mediated by clonal selection of antigen-specific lymphocytes. Adaptive immune responses are also known as **acquired immune responses**.

The **adaptor proteins** are key linkers between receptors and downstream members of signalling pathways. These proteins are functionally heterogeneous, but share an SH2 domain as the means of interacting with the phoshotyrosine residues generated by the receptor-associated tyrosine kinases. The protein known as **Vav** is an adaptor protein of this type.

The **adenoids** are **mucosal-associated lymphoid tissues** located in the nasal cavity.

The enzyme defect **adenosine deaminase deficiency** leads to the accumulation of toxic purine nucleosides and nucleotides, resulting in the death of most developing lymphocytes within the thymus. It is a common cause of severe combined immunodeficiency.

Adhesion molecules mediate the binding of one cell to other cells or to extracellular matrix proteins. Integrins, selectins, members of the immunoglobulin gene superfamily, and CD44 and related proteins are all adhesion molecules important in the operation of the immune system.

An **adjuvant** is any substance that enhances the immune response to an antigen with which it is mixed.

Adoptive immunity is immunity conferred on a naive or irradiated recipient by transfer of lymphoid cells from an actively immunized donor. This is called **adoptive transfer** or **adoptive immunization**.

Afferent lymphatic vessels drain fluid from the tissues and carry antigens from sites of infection in most parts of the body to the lymph nodes.

Affinity is the strength of binding of one molecule to another at a single site, such as the binding of a monovalent Fab fragment of antibody to a monovalent antigen. See also **avidity**.

Affinity chromatography is the purification of a substance by means of its affinity for another substance immobilized on a solid support; an antigen can be purified by affinity chromatography on a column of specific antibody molecules covalently linked to beads.

Affinity maturation refers to the increase in the affinity of the antibodies produced during the course of a humoral immune response. It is particularly prominent in secondary and subsequent immunizations.

Agammaglobulinemia: see **X-linked agammaglobulinemia**.

Agglutination is the clumping together of particles, usually by antibody molecules binding to antigens on the surfaces of adjacent particles. Such particles are said to **agglutinate**. When the particles are red blood cells, the phenomenon is called hemagglutination.

Agonist peptides are peptide antigens that activate their specific T cells, inducing them to make cytokines and to proliferate.

AIDS: see **acquired immune deficiency syndrome**.

Alleles are variants of a single genetic locus.

Allelic exclusion refers to the fact that in a heterozygous individual, only one of the alternative C-region alleles of the heavy or light chain is expressed in an immunoglobulin molecule. The term has come to be used more generally to describe the expression of a single receptor specificity in cells with the potential to express two or more receptors.

Allergens are antigens that elicit hypersensitivity or allergic reactions.

Allergic asthma is constriction of the bronchial tree due to an allergic reaction to inhaled antigen.

An **allergic r acti n** is a response to innocuous environmental antigens or allergens due to pre-existing antibody or T cells. There are various immune mechanisms of allergic reactions, but the most common is the binding of allergen to IgE antibody on mast cells that causes asthma, hay fever, and other common allergic reactions.

Allergic rhinitis is an allergic reaction in the nasal mucosa, also known as hay fever, that causes runny nose, sneezing and tears.

Allergy is the symptomatic reaction to a normally innocuous environmental antigen. It results from the interaction between the antigen and the antibody or T cells produced by earlier exposure to the same antigen.

Two individuals or two mouse strains that differ at the MHC are said to be **allogeneic**. The term can also be used for allelic differences at other loci. See also **syngeneic**; **xenogeneic**.

Rejection of grafted tissues from unrelated donors usually results from T-cell responses to **allogeneic** MHC molecules expressed by the grafted tissues.

An **allograft** is a graft of tissue from an allogeneic or non-self donor of the same species; such grafts are invariably rejected unless the recipient is immunosuppressed.

Alloreactivity describes the stimulation of T cells by MHC molecules other than self; it marks the recognition of allogeneic MHC.

Allotypes are allelic polymorphisms that can be detected by antibodies specific for the polymorphic gene products; in immunology, **allotypic** differences in immunoglobulin molecules were important in deciphering the genetics of antibodies.

An **altered peptide ligand** is a peptide, usually closely related to an agonist peptide in amino acid sequence, that induces only a partial response from T cells specific for the agonist peptide.

The **alternative pathway** of complement activation is not triggered by antibody, as is the classical pathway of complement activation, but by the binding of complement protein C3b to the surface of a pathogen; it is therefore a feature of innate immunity. The alternative pathway also amplifies the classical pathway of complement activation.

Anaphylactic shock or systemic anaphylaxis is an allergic reaction to systemically administered antigen that causes circulatory collapse and suffocation due to tracheal swelling. It results from binding of antigen to IgE antibody on connective tissue mast cells throughout the body, leading to the disseminated release of inflammatory mediators.

Anaphylatoxins are small fragments of complement proteins released by cleavage during complement activation, that recruit fluid and inflammatory cells to sites of antigen deposition. The fragments C5a, C3a, and C4a are all anaphylatoxins, listed in order of decreasing potency *in vivo*.

Peptide fragments of antigens are bound to specific MHC class I molecules by **anchor residues**, which are amino acid side chains of the peptide that bind into pockets lining the peptide-binding groove of the MHC class I molecule. Each MHC class I molecule binds different patterns of anchor residues, each called an anchor motif, giving some specificity to peptide binding. Anchor residues are less obvious for peptides that bind to MHC class II molecules.

An rgy is a state of non-responsiveness to antigen. People are said to be anergic when they cannot mount delayed-type hypersensitivity reactions to challenge antigens, whereas T and B cells are said to be anergic when they cannot respond to their specific antigen under optimal conditions of stimulation.

Antagonist peptid s are peptides, usually closely related in sequence to an agonist peptide, that inhibit the response of a cloned T-cell line specific for the agonist peptide.

An **antib dy** is a protein that binds specifically to a particular substance—its antigen. Each antibody molecule has a unique structure that enables it to bind specifically to its corresponding antigen, but all antibodies have the same overall structure and are known collectively as immunoglobulins. Antibodies are produced by plasma cells in response to infection or immunization, and bind to and neutralize pathogens or prepare them for uptake and destruction by phagocytes.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is the killing of antibody-coated target cells by cells with Fc receptors that recognize the Fc region of the bound antibody. Most ADCC is mediated by NK cells that have the Fc receptor FcγRIII or CD16 on their surface.

The **antibody repertoire** describes the total variety of antibodies that an individual can make.

An **antigen** is any molecule that can bind specifically to an antibody. Their name arises from their ability to **gen**erate **anti**bodies. However, some antigens do not, by themselves, elicit antibody production; those antigens that can induce antibody production are called immunogens.

Antigen:antibody complexes are non-covalently associated groups of antigen and antibody molecules which can vary in size from small, soluble complexes to large, insoluble complexes that precipitate out of solution; they are also known as immune complexes.

The **antigen-binding site** of an antibody is the surface of the antibody molecule that makes physical contact with the antigen. Antigen-binding sites are made up of six hypervariable loops, three from the light-chain V region and three from the heavy-chain V region.

T and B lymphocytes collectively bear on their surface highly diverse **antigen receptors** capable of recognizing a wide diversity of antigens. Each individual lymphocyte bears receptors of a single antigen specificity.

An **antigenic determinant** is the portion of an antigenic molecule that is bound by a given antibody; it is also known as an epitope.

Influenza virus varies from year to year by a process of **antigenic drift** in which point mutations of viral genes cause small differences in the structure of viral surface antigens. Periodically, influenza viruses undergo an **antigenic shift** through reassortment of their segmented genome with another influenza virus, changing their surface antigens radically. Such antigenic shift variants are not recognized by individuals immune to influenza, so when antigenic shift variants arise, there is widespread and serious disease.

Many pathogens evade the adaptive immune response by **antigenic variation** in which new antigens are displayed that are not recognized by antibodies or T cells elicited in earlier infections.

Antigen presentation describes the display of antigen as peptide fragments bound to MHC molecules on the surface of a cell; all T cells recognize antigen only when it is presented in this way.

Antigen-presenting cells are highly specialized cells that can process antigens and display their peptide fragments on the cell surface together with molecules required for lymphocyte activation. The main antigen-presenting cells for T cells are dendritic cells, macrophages, and B cells, whereas the main antigen-presenting cells for B cells are follicular dendritic cells.

Antigen pr cessing is the degradation of proteins into peptides that can bind to MHC molecules for presentation to T cells. All antigens except peptides must be processed into peptides before they can be presented by MHC molecules.

Anti-immun gl bulin antibodi s are antibodies against immunoglobulin constant domains, useful for detecting bound antibody molecules in immunoassays and other applications.

Hemat poiesis is the generation of the cellular elements of blood, including the red blood cells, leukocytes and platelets. These cells all originate from pluripotent **hemat p i tic st m cells** whose differentiated progeny divide under the influence of **hemat p i tic growth factors**.

A **hematopoietic lineage** is any developmental series of cells that derives from hematopoietic stem cells and results in the production of mature blood cells.

Hemolytic disease of the newborn, or erythroblastosis fetalis, is caused by a maternal IgG antibody response to paternal antigens expressed on fetal red blood cells. The usual target of this response is the Rh blood group antigen. The maternal anti-Rh IgG antibodies cross the placenta to attack the fetal red blood cells.

The **hemolytic plaque assay** detects antibody-forming cells by the ability of their secreted antibodies to produce a **hemolytic plaque**, an area of localized destruction of a thin layer of red blood cells around each antibody-producing cell. The antibodies secreted by the B cell are trapped by antigens on the red blood cells immediately surrounding it, and then complement is added that is triggered by the bound antibody to lyse the red blood cells.

Recombination signal sequences (RSS) flanking gene segments consist of a seven-nucleotide **heptamer** and a nine-nucleotide nonamer of conserved sequence, separated by 12 or 23 nucleotides. RSSs form the target for the site-specific recombinase that joins the gene segments.

Hereditary angioneurotic edema is the clinical name for a genetic deficiency of the C1 inhibitor of the complement system. In the absence of C1 inhibitor, spontaneous activation of the complement system can cause diffuse fluid leakage from blood vessels, the most serious consequence of which is epiglottal swelling leading to suffocation.

Individuals **heterozygous** for a particular gene have two different alleles of that gene.

An excellent model for membranous glomerulonephritis is **Heymann's nephritis**, a disease induced by injecting animals with tubular epithelial tissue.

High endothelial venules (**HEV**) are specialized venules found in lymphoid tissues. Lymphocytes migrate from blood into lymphoid tissues by attaching to and migrating across the **high endothelial cells** of these vessels.

Tolerance to injected protein antigens occurs at low or high doses of antigen. Tolerance induced by the injection of high doses of antigen is called **high-zone tolerance**, whereas tolerance produced with low doses of antigen is called low-zone tolerance.

The **hinge region** of antibody molecules is a flexible domain that joins the Fab arms to the Fc piece. The flexibility of the hinge region in IgG and IgA molecules allows the Fab arms to adopt a wide range of angles, permitting binding to epitopes spaced variable distances apart.

Histamine is a vasoactive amine stored in mast cell granules. Histamine released by antigen binding to IgE molecules on mast cells causes dilation of local blood vessels and smooth muscle contraction, producing some of the symptoms of immediate hypersensitivity reactions. Anti-histamines are drugs that counter histamine action.

Hist c mpatibility is literally the ability of tissues (Greek: *histo*) to get along with each other. It is used in immunology to describe the genetic systems that determine the rejection of tissue and organ grafts resulting from immunological recognition of histocompatibility (H) antigens.

HIV: see **human immun d ficiency virus**.

HLA, the acronym for **H**uman **L**eukocyte **A**ntigen, is the genetic designation for the human MHC. Individual loci are designated by upper-case letters, as in HLA-A, and alleles are designated by numbers, as in HLA-A*0201.

The invariant **HLA-DM** molecule in humans is involved in loading peptides onto MHC class II molecules. It is encoded in the MHC within a set of genes resembling MHC class II genes. A homologous protein in mice is called H-2M.

Hodgkin's disease is a malignant disease in which antigen-presenting cells that resemble dendritic cells seem to be the transformed cell type. **Hodgkin's lymphoma** is a form of Hodgkin's disease in which lymphocytes predominate, and it has a much better prognosis than the nodular sclerosis form of this disease in which the predominant cell type is non-lymphoid.

Homeostasis is a generic term describing the status of physiological normality. In the case of lymphocytes, homeostasis refers to an uninfected individual who has normal numbers of lymphocytes.

Cellular genes can be disrupted by **homologous recombination** with copies of the gene into which erroneous sequences have been inserted. When these exogenous DNA fragments are introduced into cells, they recombine selectively with the cellular gene through remaining regions of sequence homology, replacing the functional gene with a non-functional copy.

The **human immunodeficiency virus** (**HIV**) is the causative agent of the acquired immune deficiency syndrome (AIDS). HIV is a retrovirus of the lentivirus family that selectively infects CD4 T cells, leading to their slow depletion, which eventually results in immunodeficiency.

Humanization is a term used to describe the genetic engineering of mouse hypervariable loops of a desired specificity into otherwise human antibodies. The DNA encoding hypervariable loops of mouse monoclonal antibodies or V regions selected in phage display libraries is inserted into the framework regions of human immunoglobulin genes. This allows the production of antibodies of a desired specificity that do not cause an immune response in humans treated with them.

Humoral immunity is the antibody-mediated specific immunity made in a **humoral immune response**. Humoral immunity can be transferred to unimmunized recipients by using immune serum containing specific antibody.

Monoclonal antibodies are most commonly produced from **hybridomas**. These are hybrid cell lines formed by fusing a specific antibody-producing B lymphocyte with a myeloma cell that is selected for its ability to grow in tissue culture and an absence of immunoglobulin chain synthesis.

Hyperacute graft rejection of an allogenic tissue graft is an immediate reaction caused by natural preformed antibodies that react against antigens on the graft. The antibodies bind to endothelium and trigger the blood clotting cascade, leading to an engorged, ischemic graft and rapid loss of the organ.

Repetitive immunization to achieve a heightened state of immunity is called **hyperimmunization**.

Immune responses to innocuous antigens that lead to symptomatic reactions upon re-exposure are called **hypersensitivity reactions**. These can cause **hypersensitivity diseases** if they occur repetitively. This state of heightened reactivity to antigen is called **hypersensitivity**. Hypersensitivity reactions are classified by mechanism: type I hypersensitivity reactions involve IgE antibody triggering of mast cells; type II hypersensitivity reactions involve IgG antibodies against cell-surface or matrix antigens; type III hypersensitivity reactions involve antigen:antibody complexes; and type IV hypersensitivity reactions are T-cell mediated.

The **hyp rvariable (HV) regions** of immunoglobulin and T-cell receptor V domains are small regions that make contact with the antigen and differ extensively from one receptor to the next. Cf. **framework regions**.

ICAM: see **intercellular adhesion m l cule**.

Icc s m s are small fragments of membrane coated with immune complexes that fragment off the processes of follicular dendritic cells

in•fan•try•man (ĭn′fən-trē-mən) *n.* A soldier in the infantry.
infant school *n. Chiefly British.* A kindergarten.
in•farct (ĭn′färkt′, ĭn-färkt′) *n. Pathol.* An area of tissue that undergoes necrosis as a result of obstruction of local blood supply. [< Lat. *infarctus*, p.part. of *infarcire*, to cram : *in-*, in; see IN-[2] + *farcire*, to stuff.] — **in•farct′ed** *adj.*
in•farc•tion (ĭn-färk′shən) *n.* **1.** The formation or development of an infarct. **2.** An infarct.
in•fat•u•ate (ĭn-făch′o͞o-āt′) *tr.v.* **-at•ed, -at•ing, -ates. 1.** To inspire with unreasoning love or attachment. **2.** To cause to behave foolishly. — *adj.* (-ĭt, -āt′). Infatuated. [Lat. *infatuāre, infatuāt-* : *in-*, causative pref.; see IN-[2] + *fatuus*, foolish.]
in•fat•u•at•ed (ĭn-făch′o͞o-ā′tĭd) *adj.* Possessed by an unreasoning passion or attraction. — **in•fat′u•at′ed•ly** *adv.*
in•fat•u•a•tion (ĭ-făch′o͞o-ā′shən) *n.* **1.** A foolish, unreasoning, or extravagant passion or attraction. See Syns at **love. 2.** An object of extravagant, short-lived passion.
in•fau•na (ĭn′fô′nə) *n.* Aquatic animals that live in the substrate of a body of water. [IN-[2] + FAUNA.]
in•fea•si•ble (ĭn-fē′zə-bəl) *adj.* Not feasible; impracticable.
in•fect (ĭn-fĕkt′) *tr.v.* **-fect•ed, -fect•ing, -fects. 1.** To contaminate with a pathogen. **2.** To communicate a pathogen or disease to. **3.** To invade and produce infection in. **4.** To contaminate or corrupt. **5.** To affect in a contagious way. [ME *infecten*, to afflict with disease < Lat. *inficere, infect-*, to stain, infect : *in-*, in; see **in-*** + *facere*, to do; see **dhē-***.]
in•fec•tion (ĭn-fĕk′shən) *n.* **1.a.** Invasion by and multiplication of pathogenic microorganisms in a body tissue. **b.** An instance of being infected. **c.** An agent or a contaminated substance responsible for one's becoming infected. **d.** The pathological state resulting from having been infected. **2.** An infectious disease. **3.a.** Moral contamination or corruption. **b.** Ready communication of an emotion or attitude by contact or example.
in•fec•tious (ĭn-fĕk′shəs) *adj.* **1.** Capable of causing infection. **2.** Caused by or capable of being transmitted by infection. **3.** Easily or readily communicated: *an infectious laugh.* — **in•fec′tious•ly** *adv.* — **in•fec′tious•ness** *n.*
infectious enterohepatitis *n.* See **blackhead** 2.
infectious hepatitis *n.* See **hepatitis A.**
infectious mononucleosis *n.* An acute infectious disease caused by Epstein-Barr virus and characterized by fever, swollen lymph nodes, sore throat, and lymphocyte abnormalities.
in•fec•tive (ĭn-fĕk′tĭv) *adj.* Capable of producing infection; infectious. — **in•fec′tive•ness, in′fec•tiv′i•ty** *n.*
in•fe•lic•i•tous (ĭn′fĭ-lĭs′ĭ-təs) *adj.* **1.** Inappropriate; ill-chosen: *an infelicitous remark.* **2.** Not happy; unfortunate.
in•fe•lic•i•ty (ĭn′fĭ-lĭs′ĭ-tē) *n., pl.* **-ties. 1.** The quality or condition of being infelicitous. **2.** Something inappropriate or unpleasing. [ME *infelicite* < Lat. *infēlicitās* < *infēlix, infēlic-*, unhappy : *in-*, not; see IN-[1] + *fēlix*, happy; see **dhē(i)-***.]
in•fer (ĭn-fûr′) *v.* **-ferred, -fer•ring, -fers.** — *tr.* **1.** To conclude from evidence or premises. **2.** To reason from circumstance; surmise. **3.** To lead to as a consequence or conclusion: *"Socrates argued that a statue inferred the existence of a sculptor"* (Academy). **4.** *Usage Problem.* To hint; imply. — *intr.* To draw inferences. [Lat. *inferre*, to bring in, adduce : *in-*, in; see IN-[2] + *ferre*, to bear; see **bher-[1]***.] — **in•fer′a•ble** *adj.* — **in•fer′a•bly** *adv.* — **in•fer′rer** *n.*

Usage Note: The traditional distinction between *imply* and *infer* is a useful one. When we say that a speaker or sentence implies something, we mean that it is conveyed or suggested without being stated outright: *When the mayor said that she would not rule out a business tax increase, she implied* (not *inferred*) *that some taxes might be raised.* Inference, on the other hand, is the activity performed by a reader or interpreter in deriving conclusions that are not explicit in what is said: *When the mayor said that she would not rule out a tax increase, we inferred that she had been consulting with some new financial advisers, since her old advisers were in favor of tax reductions.*

in•fer•ence (ĭn′fər-əns) *n.* **1.a.** The act or process of deriving logical conclusions from premises known or assumed to be true. **b.** The act of reasoning from factual knowledge or evidence. **2.a.** Something inferred. **b.** *Usage Problem.* A hint or suggestion. See Usage Note at **infer.**
in•fer•en•tial (ĭn′fə-rĕn′shəl) *adj.* **1.** Of, relating to, or involving inference. **2.** Derived or capable of being derived by inference. — **in′fer•en′tial•ly** *adv.*
in•fe•ri•or (ĭn-fîr′ē-ər) *adj.* **1.** Low or lower in order, degree, or rank. **2.a.** Low or lower in quality, value, or estimation: *felt inferior to his older sibling.* **b.** Second-rate; poor. **3.** Situated under or beneath. **4.** *Bot.* Located below the perianth and other floral parts. Used of an ovary. **5.** *Anat.* Located beneath or directed downward. **6.** *Print.* Set below the normal line of type; subscript. **7.** *Astron.* **a.** Orbiting between the earth and the sun. **b.** Lying below the horizon. — *n.* **1.** A person lower in rank, status, or accomplishment than another. **2.** *Print.* An inferior character, such as the number 2 in CO_2. [ME < Lat. *inferior*, comp. of *inferus*, low. See **ndher-***.] — **in•fe′ri•or′i•ty** (-ôr′ĭ-tē, -ŏr′-) *n.*
inferiority complex *n.* A persistent sense tendency to self-diminishment.
in•fer•nal (ĭn-fûr′nəl) *adj.* **1.a.** Of or relati of the dead. **b.** Of or relating to hell. **2.** *infernal instruments of war.* **3.** Abomina OFr. < LLat. *infernālis* < *infernus*, hell derground. See **ndher-***.] — **in•fer′nal•ly**
infernal machine *n. Law.* An explosive de signed to harm or destroy.
in•fer•no (ĭn-fûr′nō) *n., pl.* **-nos. 1.** A plac gestive of hell. **2.** A place of fiery heat o hell < LLat. *infernus*. See INFERNAL.]
in•fer•tile (ĭn-fûr′tl) *adj.* **1.** Not fertile; u ren. **2.** *Biol.* Incapable of producing offs
in•fer•til•i•ty (ĭn′fər-tĭl′ĭ-tē) *n.* **1.** Absen tility. **2.** The persistent inability to conce
in•fest (ĭn-fĕst′) *tr.v.* **-fest•ed, -fest•ing,** or overrun in numbers large enough to ing, or obnoxious. **2.** To live as a parasi *infested with tapeworms.* [ME *infesten* *infester* < Lat. *infestāre* < *infestus*, h — **in′fes•ta′tion** *n.* — **in•fest′er** *n.*
in•fi•del (ĭn′fĭ-dəl, -dĕl′) *n.* **1.** An unbe a particular religion, esp. Christianity has no religious beliefs. **3.** One who d ticular doctrine, system, or principle. [M Lat. *infidēlis*, disloyal : *in-*, not; see IN- *fidēs*, faith; see **bheidh-***).]
in•fi•del•i•ty (ĭn′fĭ-dĕl′ĭ-tē) *n., pl.* -t to a sexual partner, esp. a spouse. **b.** faithfulness. **2.** Lack of loyalty. **3.** Lac
in•field (ĭn′fēld′) *n.* **1.** *Baseball.* **a.** bounded by home plate and first, sec **b.** The defensive positions of first ba base, and shortstop considered as a u inside a racetrack or running track. **3.** farmhouse.
in•field•er (ĭn′fēl′dər) *n. Baseball.* An
in•fight•ing (ĭn′fī′tĭng) *n.* **1.** Contenti ment among members or groups wi **2.** *Sports.* Fighting or boxing at close
in•fil•trate (ĭn-fĭl′trāt′, ĭn′fĭl-) *v.* **-trates.** — *tr.* **1.a.** To pass (troops, fo ly into enemy-held territory. **b.** To pe tent. **2.** To enter or take up position titiously, as for espionage. **3.** To cau to permeate by passing through in permeate (a porous substance) with gain entrance gradually or surreptit filtrates, esp. an abnormal substance ually in cells or body tissues. — **in•fi** — **in•fil′tra•tor** *n.*
in•fil•tra•tion (ĭn′fĭl-trā′shən) *n.* infiltrating. **2.** The state of being infil infiltrates.
infin. *abbr.* Infinitive.
in•fi•nite (ĭn′fə-nĭt) *adj.* **1.** Having **2.** Immeasurably great or large; *tance.* **3.** *Math.* **a.** Existing beyond arbitrarily large value. **b.** Unlimited relating to a set capable of being spondence with a proper subset of finite. — **in′fi•nite•ly** *adv.* — **in′fi**
Syns: *infinite, boundless, eter* The central meaning shared by the beginning or end": *infinite wisdom nal beauty; illimitable space; sem* at **incalculable.** ***Ant:*** *finite.*
Usage Note: *Infinite* is some terms such as *unique, absolute,* strict mathematical sense it allows comparison; one quantity cannot other. Unlike other absolute terms not permit modification by adverb In nontechnical usage *infinite* is o refer to an unimaginably large de comparison of the word is unex at **unique.**
in•fin•i•tes•i•mal (ĭn′fĭn-ĭ-tĕs′ or incalculably minute. **2.** *Math.* approaching zero as a limit. — *n.* or quantity. **2.** *Math.* An infinite NLat. *infinītēsimus*, infinite in See INFINITE.] — **in′fin•i•tes′i•m**
infinitesimal calculus *n. Math.* culus.
in•fin•i•ti•val (ĭn′fĭn-ĭ-tī′vəl)
in•fin•i•tive (ĭn-fĭn′ĭ-tĭv) *n.* A substantive while retaining that in English may be preceded *too*, or may also occur without See Usage Note at **split infin**

Host Defense Against Infection

10

Throughout this book we have examined the individual mechanisms by which the adaptive immune response acts to protect the host from pathogenic infectious agents. In the remaining five chapters, we consider how the cells and molecules of the immune system work as an integrated host defense system to eliminate or control the infectious agent and to provide long-lasting protective immunity, how failures of immune defense and unwanted immune responses can occur, and how the immune response can be manipulated to benefit the host. In this chapter, we shall examine the role of the immune system as a whole in host defense, including those innate, non-adaptive defenses that comprise early barriers to infectious disease.

The microorganisms that are encountered daily in the life of a normal healthy individual only occasionally cause perceptible disease. Most are detected and destroyed within hours by defense mechanisms that do not require a prolonged period of induction because they do not rely on the clonal expansion of antigen-specific lymphocytes: these are the mechanisms of **innate immunity**. Only if an infectious organism can breach these early lines of defense will an **adaptive immune response** ensue, with the generation of antigen-specific effector cells that specifically target the pathogen, and memory cells that prevent subsequent infection with the same microorganism.

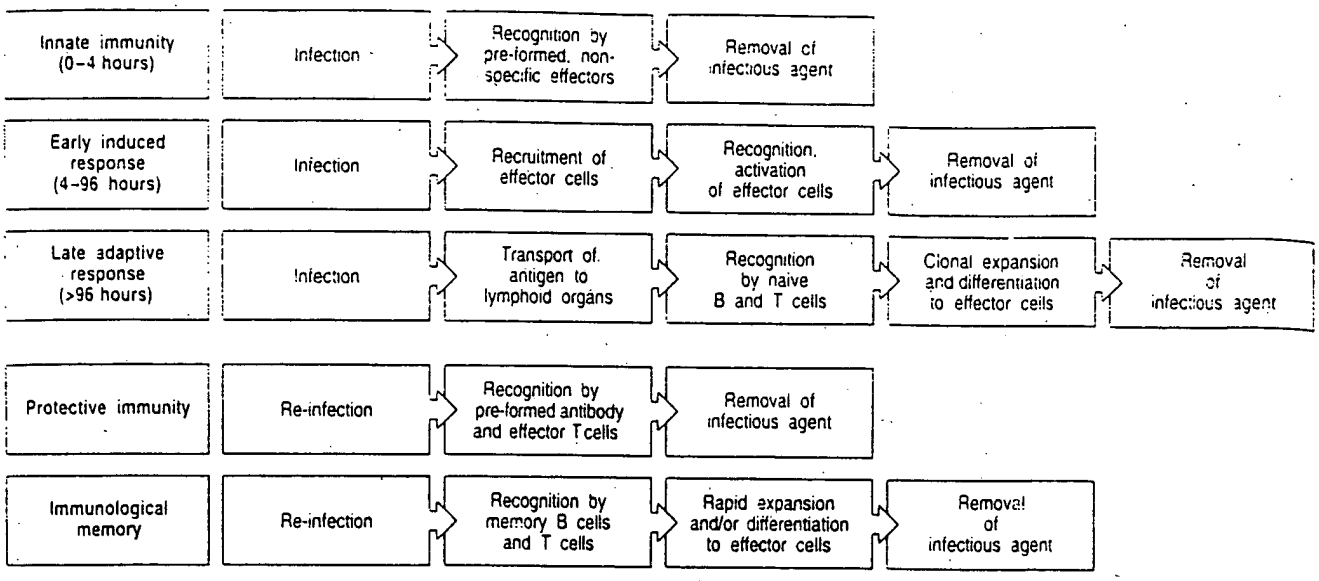


Fig. 10.1 The response to an initial infection occurs in three phases. The effector mechanisms that remove the infectious agent (eg phagocytes, NK cells, complement) are similar or identical in each phase but the recognition mechanisms differ. Adaptive immunity occurs late, because rare antigen-specific cells must undergo clonal expansion before they can differentiate into effector cells. After an adaptive immune response to a pathogen, the response to re-infection is much more rapid: pre-formed antibodies and effector cells act immediately on the pathogen, and immunological memory speeds a renewed adaptive response.

In the preceding two chapters, we have discussed how an adaptive immune response is induced, and how pathogens are eliminated or controlled by the effector cells generated in such a response. Here these mechanisms will be set in the broader context of the entire array of mammalian host defenses against infection, beginning with the innate immune mechanisms that successfully prevent most infections from becoming established. This type of immunity also has an essential role in inducing the subsequent adaptive response to those infections that do overcome the first lines of defense.

The time course of the different phases of an immune response is summarized in Fig. 10.1. The innate immune mechanisms act immediately, and are followed some hours later by **early induced responses**, which can be activated by infection but do not generate lasting protective immunity. These early phases help to keep infection under control while the antigen-specific lymphocytes of the adaptive immune response are activated. Moreover, cytokines produced during these early phases have an important role in shaping the subsequent development of the adaptive immune response and can determine whether the response is predominantly T-cell mediated or humoral. Several days are required for the clonal expansion and differentiation of naive lymphocytes into effector T cells and antibody-secreting B cells that can target the pathogen for elimination. During this period, specific immunological memory is also established: this ensures a rapid re-induction of antibody and antigen-specific effector T cells on subsequent encounters with the same pathogen, thus providing long-lasting protection against re-infection. In this chapter we shall learn how the different phases of host defense are orchestrated in space and time, and how changes in specialized cell-surface molecules guide lymphocytes to the appropriate site of action at different stages of the immune response.

Infection and innate immunity.

Microorganisms that cause pathology in humans and animals enter the body at different sites and produce disease by a variety of mechanisms. Such invasions are initially countered, in all vertebrates, by innate defense mechanisms that pre-exist in all individuals and act within minutes of infection. Only when the innate host defenses are by-passed, evaded, or overwhelmed is an induced or adaptive immune response required, and even then the same effector mechanisms that operate in innate immunity are ultimately harnessed to eliminate the pathogen. In this section we shall describe briefly the infectious strategies of microorganisms, before examining the innate host defenses that, in most cases, prevent infection from becoming established.

10-1 The infectious process can be divided into several distinct phases.

The process of infection can be broken down into stages, each of which can be blocked by different defense mechanisms. Before these are deployed, an infection must be established by infectious particles shed by an infected individual. The number, route, mode of transmission, and stability of an infectious agent outside the host determine its infectivity. Some pathogens, such as anthrax, are spread by spores that are highly resistant to heat and drying, whereas others, such as the human immunodeficiency virus, are spread only by the exchange of body fluids such as blood because they are unable to survive as isolated infectious agents.

Although the body is constantly exposed to infectious agents, infectious disease is fortunately quite rare. The epithelial surfaces of the body serve as an efficient barrier to most microorganisms and those that enter are efficiently removed by innate immune mechanisms. Only when a microorganism has crossed an epithelial barrier and established a site of infection does infectious disease occur, and little pathology will be caused unless the agent is able to spread. Extracellular pathogens spread by direct extension of the infectious center, or through the lymphatics or the bloodstream. Usually, spread through the bloodstream occurs only after the lymphatic system has been overwhelmed by the burden of infectious agent. Obligate intracellular pathogens must spread from cell to cell; they do so either by direct transmission from one cell to the next or by release into the extracellular fluid and reinfection of both adjacent and distant cells.

Most infectious agents show a significant degree of host specificity, causing disease only in one or a few related species. What determines host specificity for each agent is not known but the requirement for attachment to a particular cell-surface molecule is one factor, and other interactions with host cells are commonly needed to support replication. The molecular mechanism of host specificity is an area of intense research interest known as molecular pathogenesis.

Although most microorganisms are repelled by innate host defenses, an initial infection once established generally leads to perceptible disease followed by an effective host adaptive immune response. A cure involves the clearance of both extracellular infectious particles and intracellular residues of infection. After many infections there is little or no residual pathology after an effective primary response. In some cases, however, the infection or the response to it

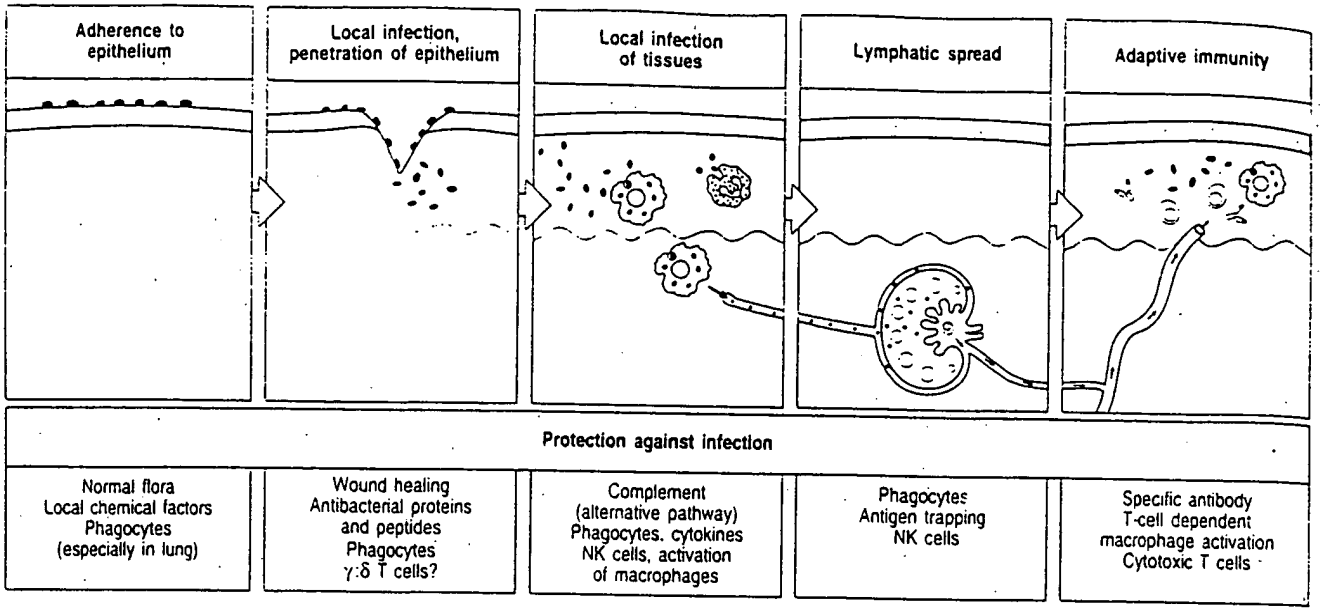


Fig. 10.2 Infections and the responses to them can be divided into a series of stages. These are illustrated here for an infectious microorganism entering across an epithelium, the commonest route of entry. The infectious organism must first adhere to the epithelial cells and then cross the epithelium. A local non-adaptive response helps contain the infection and delivers antigen to local lymph nodes, leading to adaptive immunity and clearance of the infection. The role of γ:δ T cells is uncertain, as indicated by the question mark.

cause significant tissue damage. Also, some pathogens such as cytomegalovirus or *Mycobacterium tuberculosis* are contained but not completely cleared. Thus, when the adaptive immune response is later weakened, as it is in acquired immune deficiency syndrome (AIDS), these diseases reappear.

In addition to clearance of the infectious agent, an effective adaptive immune response prevents re-infection. For some infectious agents, this protection is essentially absolute, whereas for others infection is reduced or attenuated upon re-exposure. The progress of an infection is illustrated in Fig. 10.2, which summarizes the defense mechanisms activated at each stage, each of which will be described in detail in the course of this chapter.

10-2 Infectious diseases are caused by diverse living agents that replicate in their hosts.

The agents that cause disease fall into five groups: viruses, bacteria, fungi, protozoa, and helminths (worms). Protozoa and worms are usually grouped together as parasites, and are the subject of the discipline of parasitology, whereas viruses, bacteria, and fungi are the subject of microbiology. In Fig. 10.3, the common classes of microorganisms and parasites are listed with typical examples of each. The remarkable variety of these pathogens has required potential hosts to develop two crucial features of adaptive immunity. First, the need to recognize a wide range of different pathogens has driven the development of receptors on B and T cells of equal or greater diversity. Second, the distinct habitats and life cycles of pathogens have to be countered by a range of distinct effector mechanisms. The characteristic features of each pathogen are its mode of transmission, its mechanism of replication, its pathogenesis or the means by which it causes disease, and the response it elicits. We will focus here on the immune responses to these pathogens.

Some common causes of disease in humans			
Viruses	DNA viruses	Adenoviruses	Human adenoviruses (eg types 3, 4, and 7)
		Herpesviruses	Herpes simplex, varicella zoster, Epstein–Barr virus, cytomegalovirus, Kaposi's sarcoma
		Poxviruses	Vaccinia virus
		Parvoviruses	Human parvovirus
		Papovaviruses	Papilloma virus
		Hepadnaviruses	Hepatitis B virus
	RNA viruses	Orthomyxoviruses	Influenza virus
		Paramyxoviruses	Mumps, measles, respiratory syncytial virus
		Coronaviruses	Common cold viruses
		Picornaviruses	Polio, coxsackie, hepatitis A, rhinovirus
		Reoviruses	Rotavirus, reovirus
		Togaviruses	Rubella, arthropod-borne encephalitis
		Flaviviruses	Arthropod-borne viruses, (yellow fever, dengue fever)
		Arenaviruses	Lymphocytic choriomeningitis, Lassa fever
		Rhabdoviruses	Rabies
		Retroviruses	Human T-cell leukemia virus, HIV
Bacteria	Gram +ve cocci	Staphylococci	*Staphylococcus aureus*
		Streptococci	*Streptococcus pneumoniae, S. pyogenes*
	Gram –ve cocci	Neisseriae	*Neisseria gonorrhoeae, N. meningitidis*
	Gram –ve bacilli		*Corynebacteria, Bacillus anthracis, Listeria monocytogenes*
	Gram –ve bacilli		*Salmonella, Shigella, Campylobacter, Vibrio, Yersinia, Pasteurella, Pseudomonas, Brucella, Haemophilus, Legionella, Bordetella*
	Anaerobic bacteria	Clostridia	*Clostridium tetani, C. botulinum, C. perfringens*
	Spirochetes		*Treponema pallidum, Borrelia burgdorferi, Leptospira interrogans*
	Mycobacteria		*Mycobacterium tuberculosis, M. leprae, M. avium*
	Rickettsias		*Rickettsia prowazeki*
	Chlamydias		*Chlamydia trachomatis*
	Mycoplasmas		*Mycoplasma pneumoniae*
Fungi			*Candida albicans, Cryptococcus neoformans, Aspergillus, Histoplasma capsulatum, Coccidioides immitis, Pneumocystis carinii*
Protozoa			*Entamoeba histolytica, Giardia, Leishmania, Plasmodium, Trypanosoma, Toxoplasma gondii, Cryptosporidium*
Worms	Intestinal		*Trichuris trichiura, Trichinella spiralis, Enterobius vermicularis, Ascaris lumbricoides, Ancylostoma, Strongyloides*
	Tissues		*Filaria, Onchocerca volvulus, Loa loa, Dracunculus medinensis*
	Blood, liver		*Schistosoma, Clonorchis sinensis*

Fig. 10.3 A variety of microorganisms can cause disease. Pathogenic organisms are of five main types: viruses, bacteria, fungi, protozoa, and worms. Some common pathogens in each group are listed in the column on the right.

Infectious agents can grow in various body compartments, as shown schematically in Fig. 10.4. We have already seen that two major compartments can be defined—intracellular and extracellular. Intracellular pathogens must invade host cells in order to replicate, and must either be prevented from entering cells or detected and eliminated once they have done so. Such pathogens can be subdivided further into those that replicate freely in the cell, such as viruses and certain bacteria (species of *Chlamydia* and *Rickettsia* as well as *Listeria*), and those such as the mycobacteria, that replicate in intracellular vesicles. Many microorganisms replicate in extracellular spaces, either within the body or on the surface of epithelia. Extracellular bacteria are usually susceptible to killing by phagocytes and thus have developed means to resist engulfment. The encapsulated Gram-positive cocci, for instance, grow in extracellular spaces and resist phagocytosis by means of their polysaccharide capsule; if this mechanism of resistance is overcome by opsonization, they are readily killed after ingestion by phagocytic cells.

Different infectious agents cause markedly different diseases, reflecting the diverse processes by which they damage tissues (Fig. 10.5). Many extracellular pathogens cause disease by releasing toxic products or toxins (see Fig. 9.20). Intracellular infectious agents frequently cause disease by damaging the cells that house them. The immune response to the infectious agent can itself be a major cause of pathology in several diseases (see Fig. 10.5). The pathology caused by a particular infectious agent also depends on the site in which it grows, so that *Streptococcus pneumoniae* in the lung causes pneumonia, whereas in the blood it causes a rapidly fatal systemic illness.

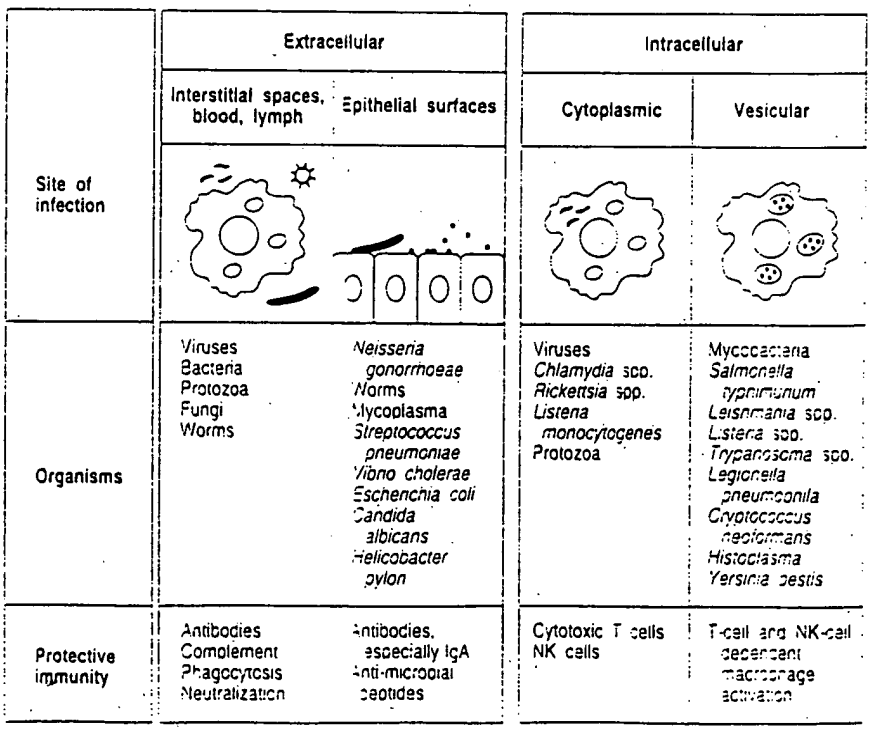


Fig. 10.4 Pathogens can be found in various compartments in the body, where they must be combated by different host defense mechanisms. Virtually all pathogens have an extracellular phase where they are vulnerable to antibody-mediated effector mechanisms. However, intracellular phases are not accessible to antibody, and these are attacked by T cells.

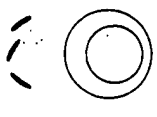
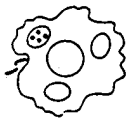
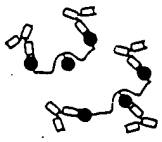
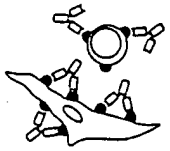
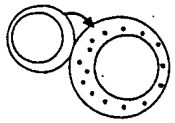
	Direct mechanisms of tissue damage by pathogens			Indirect mechanisms of tissue damage by pathogens		
	Exotoxin production	Endotoxin	Direct cytopathic effect	Immune complexes	Anti-host antibody	Cell-mediated immunity
Pathogenic mechanism						
Infectious agent	*Streptococcus pyogenes* *Staphylococcus aureus* *Corynebacterium diphtheriae* *Clostridium tetani* *Vibrio cholerae*	*Escherichia coli* *Haemophilus influenzae* *Salmonella typhi* *Shigella* *Pseudomonas aeruginosa* *Yersinia pestis*	Variola Varicella-zoster Hepatitis B virus Polio virus Measles virus Influenza virus Herpes simplex virus	Hepatitis B virus Malaria *Streptococcus pyogenes* *Treponema pallidum* Most acute infections	*Streptococcus pyogenes* *Mycoplasma pneumoniae*	*Mycobacterium tuberculosis* *Mycobacterium leprae* Lymphocytic choriomeningitis virus *Borrelia burgdorferi* *Schistosoma mansoni* Herpes simplex virus
Disease	Tonsilitis, scarlet fever Boils, toxic shock syndrome, food poisoning Diphtheria Tetanus Cholera	Gram-negative sepsis Meningitis, pneumonia Typhoid Bacillary dysentery Wound infection Plague	Smallpox Chickenpox, shingles Hepatitis Poliomyelitis Measles, subacute sclerosing panencephalitis Influenza Cold sores	Kidney disease Vascular deposits Glomerulonephritis Kidney damage in secondary syphilis Transient renal deposits	Rheumatic fever Hemolytic anemia	Tuberculosis Tuberculoid leprosy Aseptic meningitis Lyme arthritis Schistosomiasis Herpes stromal keratitis

Fig. 10.5 Pathogens can damage tissues in a variety of different ways. The mechanisms of damage, representative infectious agents, and the common name of the disease associated with each are shown. Exotoxins are released by microorganisms and act at the surface of host cells, for example by binding receptors. Endotoxins, which are intrinsic components of microbial structure, trigger phagocytes to release cytokines that produce local or systemic symptoms. Many pathogens are cytopathic, directly damaging the cells they infect. Finally, adaptive immune responses to the pathogen can generate antigen:antibody complexes that can, in turn, activate neutrophils and macrophages, antibodies that cross-react with host tissues, or T cells that kill infected cells, all with some potential to damage the host's tissues. In addition, neutrophils, the most abundant cells early in infection, release many proteins and small-molecule inflammatory mediators that both control infection and cause tissue damage (not shown).

10-3 Surface epithelia make up a natural barrier to infection.

Our body surfaces are defended by epithelia, which provide a physical barrier between the internal milieu and the external world containing pathogens. These epithelia comprise the skin and the linings of the body's tubular structures, such as the gastrointestinal, respiratory, and genito-urinary tracts. Infections occur only when the pathogen can colonize or cross over these barriers. The importance of epithelia in protection against infection is obvious when the barrier is breached, as in wounds and burns, where infection is a major cause of mortality and morbidity. People with defective secretion of mucus or inhibition of ciliary movement, where bacteria can colonize the epithelial surface, frequently develop lung infections. In the absence of wounding or disruption, pathogens normally cross epithelial barriers by adhering to molecules on mucosal epithelial cells. This specific attachment allows the pathogen to infect the epithelial cell, or to damage it so that the epithelium can be crossed.

Epithelial barriers to infection	
Mechanical	Epithelial cells joined by tight junctions Longitudinal flow of air or fluid across epithelium Movement of mucus by cilia
Chemical	Fatty acids (skin) Enzymes: lysozyme (saliva, sweat, tears), pepsin (gut) Low pH (stomach) Antibacterial peptides: cryptidins (intestine)
Microbiological	Normal flora compete for nutrients and attachment to epithelium and can produce antibacterial substances

Fig. 10.6 Surface epithelia provide mechanical, chemical, and microbiological barriers to infection.

Our surface epithelia are more than mere physical barriers to infection: they also produce chemical substances that are microbicidal or inhibit microbial growth (Fig. 10.6). For instance, the acid pH of the stomach and digestive enzymes of the upper gastrointestinal tract make a substantial chemical barrier to infection. Antibacterial peptides called cryptidins are made by Paneth cells, which are resident in the base of the crypts in the small intestine beneath the epithelial stem cells. Furthermore, most epithelia are associated with a normal flora of non-pathogenic bacteria that compete with pathogenic microorganisms for nutrients and for attachment sites on cells. The normal flora can also produce antimicrobial substances, such as the colicins (antibacterial proteins made by *Escherichia coli*) that prevent colonization by other bacteria. When the non-pathogenic bacteria are killed by antibiotic treatment, pathogenic microorganisms frequently replace them and cause disease.

When a pathogen crosses an epithelial barrier and begins to replicate in the tissues of the host, the host's defense mechanisms are required to remove the pathogen. The first phase of host defense depends upon the cells and molecules that mediate innate immunity.

10-4 The alternative pathway of complement activation provides a non-adaptive first line of defense against many microorganisms.

The alternative pathway of complement activation can proceed on many microbial surfaces in the absence of specific antibody (see Fig. 9.40). In this way, it triggers the same antimicrobial actions as the classical pathway without the delay of 5–7 days required for antibody production, and can be regarded as an innate humoral response.

The reaction cascade of the alternative pathway of complement activation is shown schematically in Fig. 10.7. C3 is abundant in plasma, and C3b is produced at a significant rate by spontaneous cleavage (also known as 'tickover') due to the hydrolysis of the thioester bond in C3 to form C3(H_2O) which has an altered conformation, allowing binding of the plasma protein factor B. The binding of B by C3(H_2O) allows a plasma protease called factor D to cleave factor B to Ba and Bb. The C3(H_2O)Bb complex is a C3 convertase that can cleave many molecules of C3 to C3a and C3b. Although much of this C3b is inactivated by hydrolysis, some attaches covalently, through its reactive thioester group, to the surfaces of host cells or to pathogens. C3b bound in this way is able to bind factor B, inducing its cleavage by factor D to yield the small fragment Ba and the active protease Bb. When this occurs on the surface of a host cell, as we learned in Chapter 9, the C3b.Bb complex is

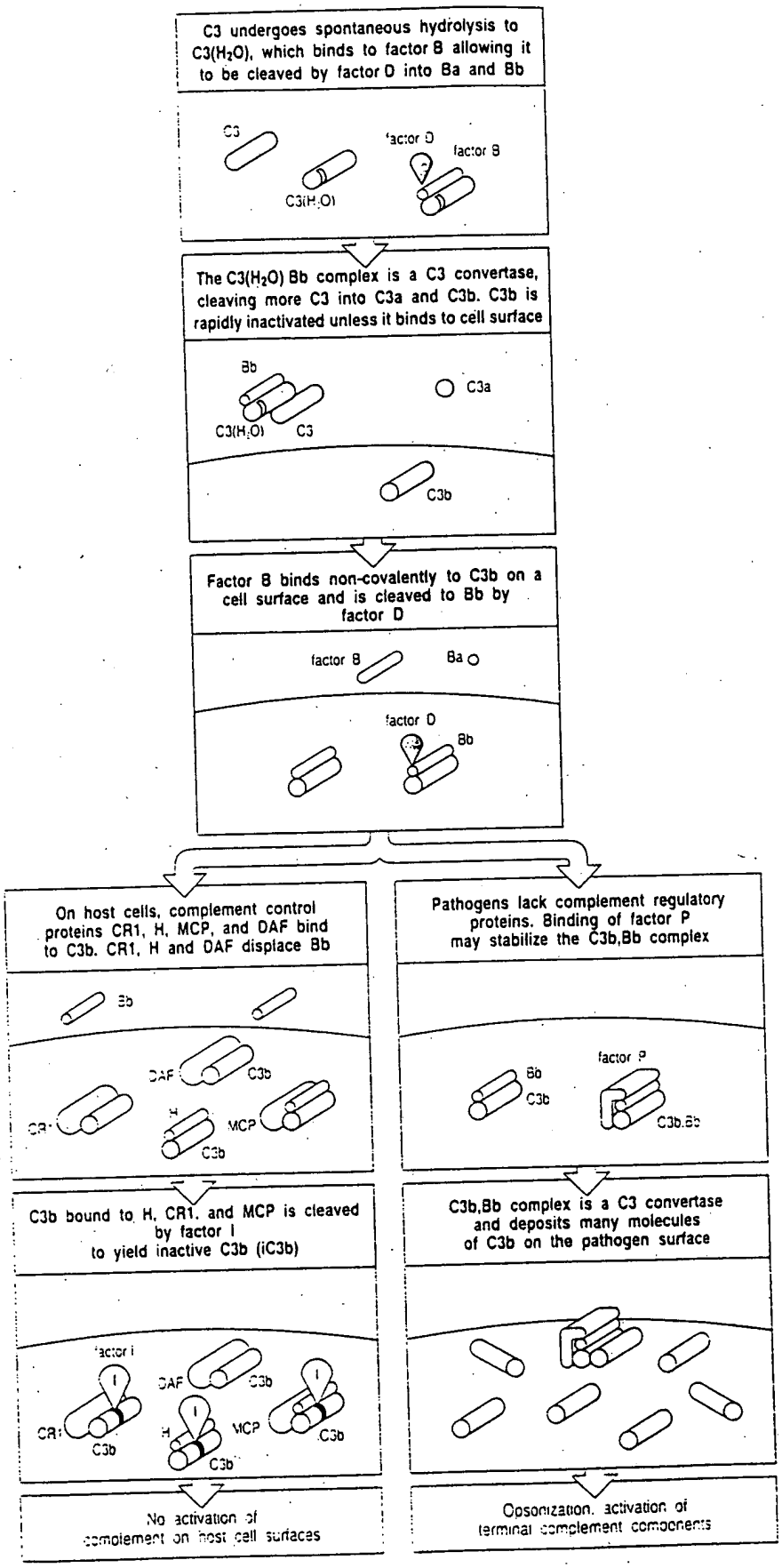


Fig. 10.7 Complement activated by the alternative pathway attacks pathogens while sparing host cells, which are protected by complement regulatory proteins. The complement component C3 is cleaved spontaneously in plasma to give $C3(H_2O)$, which binds factor B and enables this to be cleaved by factor D (top panel). The resulting soluble C3 convertase cleaves C3 to give C3a and C3b, which can attach to host or pathogen (second panel). It binds factor B, which in turn is rapidly cleaved by factor D to Bb, which remains bound to C3b to form a C3 convertase, and Ba, which is released (third panel). If C3b,Bb forms on the surface of host cells (bottom left panels) it is rapidly inactivated by complement regulatory proteins expressed by the host cell: complement receptor 1 (CR1), decay accelerating factor (DAF), and membrane co-factor of proteolysis (MCP). Host cell surfaces also favor binding of factor H from plasma. CR1, DAF, and factor H displace Bb from C3b, and CR1, MCP, and factor H catalyze the cleavage of bound C3b by factor I to produce inactive C3b (known as iC3b). Bacterial surfaces (bottom right panels) do not express complement regulatory proteins and favor binding of factor P (properdin), which stabilizes the C3b,Bb convertase activity. This convertase is the equivalent of C4b,C2b of the classical pathway (see Fig. 10.8) and initiates the cleavage of further molecules of C3 leading to opsonization by C3b and the generation of $C3b_2Bb$, the alternative pathway C5 convertase, leading to activation of the terminal complement components.

prevented from initiating further activation steps by the cell-surface proteins CR1 (complement receptor 1), DAF (decay-accelerating factor), and MCP (membrane co-factor of proteolysis), and by the plasma protein factor H. CR1 and DAF are membrane-associated molecules, whereas factor H has affinity for the terminal sialic acids of host cell membrane glycoproteins and thus also binds to the surfaces of host cells. All these bind to C3b, displacing Bb and thus preventing the next step in the activation pathway. In addition, factor H, CR1, and MCP render C3b susceptible to cleavage by factor I, a serine protease that circulates in active form and cleaves C3b first into iC3b and then further to C3dg, thus permanently inactivating it (see Fig. 10.7).

Microbial cells lack the protective proteins CR1, MCP, and DAF, and also the sialic acids that allow factor H to bind preferentially to C3b on the surface of host cells. Consequently, the C3b,Bb complexes formed on the surface of a microorganism are not dissociated and function as active C3 convertases. It seems that microbial cells also favor the binding of a positive regulatory component of the alternative pathway known as **properdin**, or factor P, which augments activation by binding to C3b,Bb complexes and stabilizing them, preventing their dissociation by factor H and subsequent cleavage by factor I. The stabilized C3 convertase then acts in the same way as the C3 convertase of the classical pathway (see Section 9-25) and converts large numbers of free C3 molecules to C3b, which coats the adjacent surface, and C3a, which mediates local inflammation. C3b and its derivative iC3b opsonize the pathogen for uptake by complement receptors expressed on phagocytic cells. The derivative iC3b can be further cleaved to C3dg, which also remains bound to the pathogen. C3dg is the ligand for CD21, which forms part of the B-cell co-receptor complex and can engage this co-receptor to increase B-cell signaling 100- to 1000-fold in response to specific antigen.

Some molecules of C3b bind to the existing C3b,Bb complex to form $C3b_2$,Bb, the alternative pathway C5 convertase. This binds and cleaves C5, initiating the lytic pathway and releasing the potent inflammatory peptide C5a (see Section 9-29). Once initiated, the alternative pathway can promote its own feedback amplification, with bound C3b binding more molecules of factor B, further increasing C3 and C5 convertase activity on the pathogen surface. Thus the principal effector molecules generated by the alternative pathway of complement activation are the same as those generated by the classical pathway in an adaptive immune response: C3b and its derivatives opsonize pathogens for uptake and destruction by phagocytes, whereas C5a and C3a stimulate the influx of more phagocytes to the site of infection.

Not all microbial surfaces allow activation of the alternative pathway, and it is not clear what distinguishes surfaces that allow the cascade to proceed from those that do not. Some bacterial surfaces have high levels of sialic acid residues, like the surfaces of vertebrate cells, and are therefore more resistant to attack by the alternative pathway than most bacteria, which do not have surface sialic acid. These bacteria favor binding of factor H, which displaces factor B from the bound C3b and makes C3b susceptible to inactivation by factor I.

Only two events in the classical pathway of complement activation are not exactly homologous to the equivalent steps in the alternative pathway: the first is the initial cleavage that, in the classical pathway, deposits C4b on the bacterial surface; the second is the cleavage that generates C2b, the active protease of the classical pathway C3 convertase. Both of these steps are mediated in the classical pathway by activation of C1s by bound antibody; in the alternative pathway, C3 is activated spontaneously, whereas factor B is activated by the plasma protein factor D.

Step in pathway	Protein serving function in pathway			Relationship
	Alternative (innate)	Lectin	Classical	
Initiating serine protease	D	MASP	C1s	Homologous (C1s and MASP)
Covalent binding to cell surface	C3b	C4b		Homologous
C3,C5 convertase	Bb	C2b		Homologous
Control of activation	CR1 H	CR1 C4bp		Identical Homologous
Opsonization	C3b			Identical
Initiation of effector pathway	C5b			Identical
Local inflammation	C5a, C3a			Identical

Fig. 10.8 There is a close relationship between the factors of the alternative, lectin-mediated, and classical pathways of complement activation. Most of the factors are either identical or the products of genes that have duplicated and then diverged in sequence. The proteins C4 and C3 are homologous and contain the unstable thioester bond by which their large fragments, C4b and C3b, bind covalently to membranes. The genes encoding proteins C2 and B are adjacent in the class III region of the MHC and arose by gene duplication. Factor H, CR1, and C4bp regulatory proteins share a repeat sequence common to many complement regulatory proteins. The greatest divergence between the pathways is in their initiation: in the classical pathway the C1 complex serves to convert antibody binding into enzyme activity on a specific surface; in the lectin-mediated pathway, mannan-binding-lectin (MBL) associates with a serine protease, forming MBL-associated serine protease (MASP), to serve the same function; whereas in the alternative pathway this enzyme activity is provided by factor D.

The alternative pathway of complement activation thus illustrates the general principle that most of the immune effector mechanisms that can be activated by the adaptive immune response can also be induced in a non-clonal fashion as part of the early, non-adaptive host response against infection. It is almost certain that the adaptive response evolved by adding specific recognition to the original non-adaptive system. This is illustrated particularly clearly in the complement system, because here the components are defined, and the functional homologs can be seen to be evolutionarily related (Fig. 10.8).

10-5 Phagocytes provide innate cellular immunity in tissues and initiate host-defense responses.

Macrophages mature continuously from circulating monocytes (see Fig. 1.3) and leave the circulation to migrate into tissues throughout the body. They are found in especially large numbers in connective tissue, in association with the gastrointestinal tract, in the lung (where they are found in the interstitium and the alveoli), along certain blood vessels in the liver (where they are known as Kupffer cells), and throughout the spleen where they serve to remove senescent red blood cells. The second major family of phagocytes—the neutrophils, or polymorphonuclear neutrophilic leukocytes (PMNs, or polys)—are produced and lost in large numbers each day. Both these phagocytic cells have a key role in all phases of host defense. In addition to engulfing opsonized particles coated with antibodies and/or complement, they can recognize and ingest many pathogens directly. Indeed, the same complement receptors by which they engulf opsonized particles recognize various microbial constituents. For example, the leukocyte integrins CD11b/CD18 (also known as CR3 or Mac-1) and CD11c/CD18 (CR4) are able to recognize several microbial substances, including bacterial lipopolysaccharide (LPS), the lipophosphoglycan of *Leishmania*, the filamentous hemagglutinin of *Bordetella*, and structures on yeasts such as *Candida* and *Histoplasma*. Tissue macrophages and neutrophils also have on their surface other receptors able to recognize components common to many pathogens. These receptors include the macrophage mannose receptor, which is found on macrophages

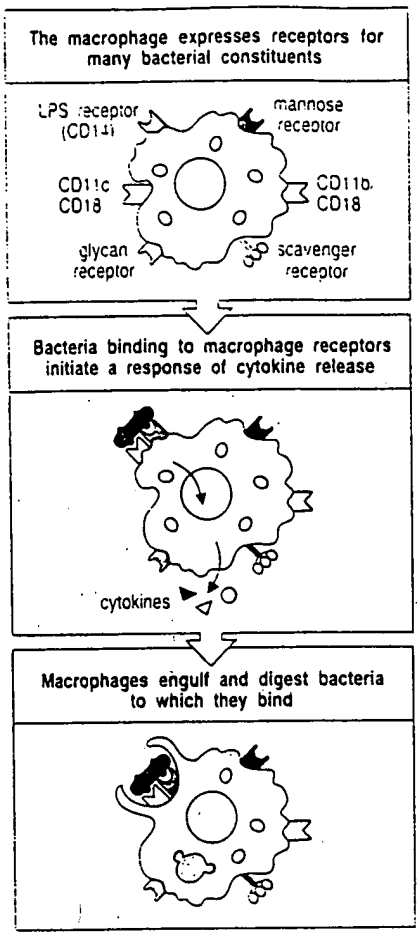


Fig. 10.9 Phagocytes bear several different receptors that recognize microbial components and induce phagocytosis and the release of cytokines. The figure illustrates this for two such receptors, CD14 and CD11c/CD18 (CR4), both of which are specific for bacterial lipopolysaccharide (LPS).

but not on monocytes or neutrophils, the scavenger receptor, which binds many sialidated ligands, and CD14, an LPS-binding molecule found predominantly on monocytes and macrophages (Fig. 10.9).

When pathogens cross an epithelial barrier, they are immediately recognized by phagocytes in the subepithelial connective tissues, with three important consequences. The first is the trapping, engulfment, and destruction of the pathogen by tissue macrophages and migrating neutrophils. In addition to being phagocytic, macrophages and neutrophils have granules that contain enzymes, proteins, and peptides that can mediate an intracellular antibacterial response. This innate cellular immune response is immediate and can be sufficient to prevent an infection from becoming established, even after a microbe has crossed an epithelial barrier. Indeed, the great cellular immunologist Elie Metchnikoff believed that the innate response of macrophages and neutrophils encompassed all host defense. For a microbe to become pathogenic, it must devise strategies of avoiding engulfment by phagocytes or, like the mycobacteria, devise ways of growing inside the phagosome; many extracellular bacteria coat themselves with a thick polysaccharide capsule that is not recognized by any phagocyte receptor. Even without such devices, if sufficient bacteria enter the body and simply overwhelm the innate host defenses, they can establish a focus of infection.

The second important effect of the interaction of phagocytes with pathogens is the secretion of cytokines by the phagocyte. It is thought that the pathogen induces cytokine secretion by binding to the same receptors used for engulfment. Cytokines are an important component of the next phase of host defense, which comprises a series of induced but non-adaptive responses, as discussed in the next part of this chapter. Cytokine release is also induced by the small peptides released from the complement cascade.

Finally, as we learned in Chapter 8, receptors on macrophages (but not neutrophils) have an important role in antigen uptake and processing, and signals transmitted by these receptors are likely to be responsible for inducing the expression of co-stimulatory molecules that allow the macrophage to function as a professional antigen-presenting cell. Thus, macrophages are important in the induction of the adaptive immune response, and their released cytokines have an additional role in determining the form of the adaptive immune response, as we shall see in the third part of this chapter.

Summary

The mammalian body is susceptible to infection by many pathogens, which must first make contact with the host and then establish a focus of infection in order to cause disease. These pathogens differ greatly in their lifestyles and means of pathogenesis; this requires an equally diverse set of defensive responses from the host immune system. The first phase of host defense is called innate immunity, and consists of those mechanisms that are present and ready to attack an invader at any time. The epithelial surfaces of the body keep pathogens out as a first line of defense, and many viruses and bacteria can enter tissues only through specialized cell-surface interactions. Bacteria that overcome this barrier are faced with two immediate lines of defense. First, they are subject to humoral attack by the alternative pathway of complement activation, which is spontaneously active in plasma and can opsonize or destroy bacteria while sparing host cells, which are protected by complement regulatory proteins. Second, they can be directly recognized and engulfed by phagocytic macrophages and neutrophils with receptors for common bacterial components.

Innate immunity involves the direct engagement of an effector mechanism by the pathogen, acts immediately on contact with it, and is unaltered in its ability to resist a subsequent challenge. This distinguishes innate immunity from the induced responses that we shall consider next and from the adaptive immune response that provides long-lasting protection against re-infection.

Non-adaptive host responses to infection.

The activation of complement by the alternative pathway and the engulfment of microorganisms by phagocytes occur in the early hours of local infection. If the microorganism evades or overwhelms these innate defenses, the infection can still be contained by a second wave of responses involving the activation of a variety of humoral and cell-mediated effector mechanisms that are strikingly similar to those discussed in Chapters 8 and 9. These are the early induced responses. Unlike the adaptive response, these responses to pathogens involve recognition mechanisms that are based on relatively invariant receptors, and they do not lead to the lasting protective immunity against the inducing pathogen that is the hallmark of adaptive immunity. Instead, as we shall see, the same response is usually made to all pathogens of a given class.

The early induced but non-adaptive responses are important for two main reasons. First, they can repel a pathogen or, more often, hold it in check until an adaptive immune response can be mounted. The early responses occur rapidly, because they do not require clonal expansion, whereas adaptive responses have a latent period of clonal expansion before the proliferating lymphocytes mature into effector cells capable of eliminating an infection. Second, these early responses influence the adaptive response in several ways, as we shall see when we consider this later phase of host defense in the next part of this chapter.

10-6 The innate immune response produces inflammatory mediators that recruit new phagocytic cells to local sites of infection.

One important function of the innate immune response is to recruit more phagocytic cells and effector molecules to the site of the infection through the release of a battery of cytokines and other inflammatory mediators that have profound effects on subsequent events. The cytokines secreted by phagocytes in response to infection are a structurally diverse group of molecules and include interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α). All have important local and systemic effects, which are summarized in Fig. 10.10. Phagocytes also release other proteins with potent local effects, such as the enzymes plasminogen activator and phospholipase.

Phagocytes release a variety of other molecules in response to infectious agents, including toxic oxygen radicals, peroxides, nitric oxide (NO), and lipid mediators of inflammation such as prostaglandins, leukotrienes—particularly leukotriene B4 (LTB4)—and platelet-activating factor (PAF). In addition, the activation of complement by infectious agents contributes the inflammatory mediators C5a and the less potent C3a. As well as being an inflammatory mediator in its own right, C5a is also able to activate mast cells, causing them

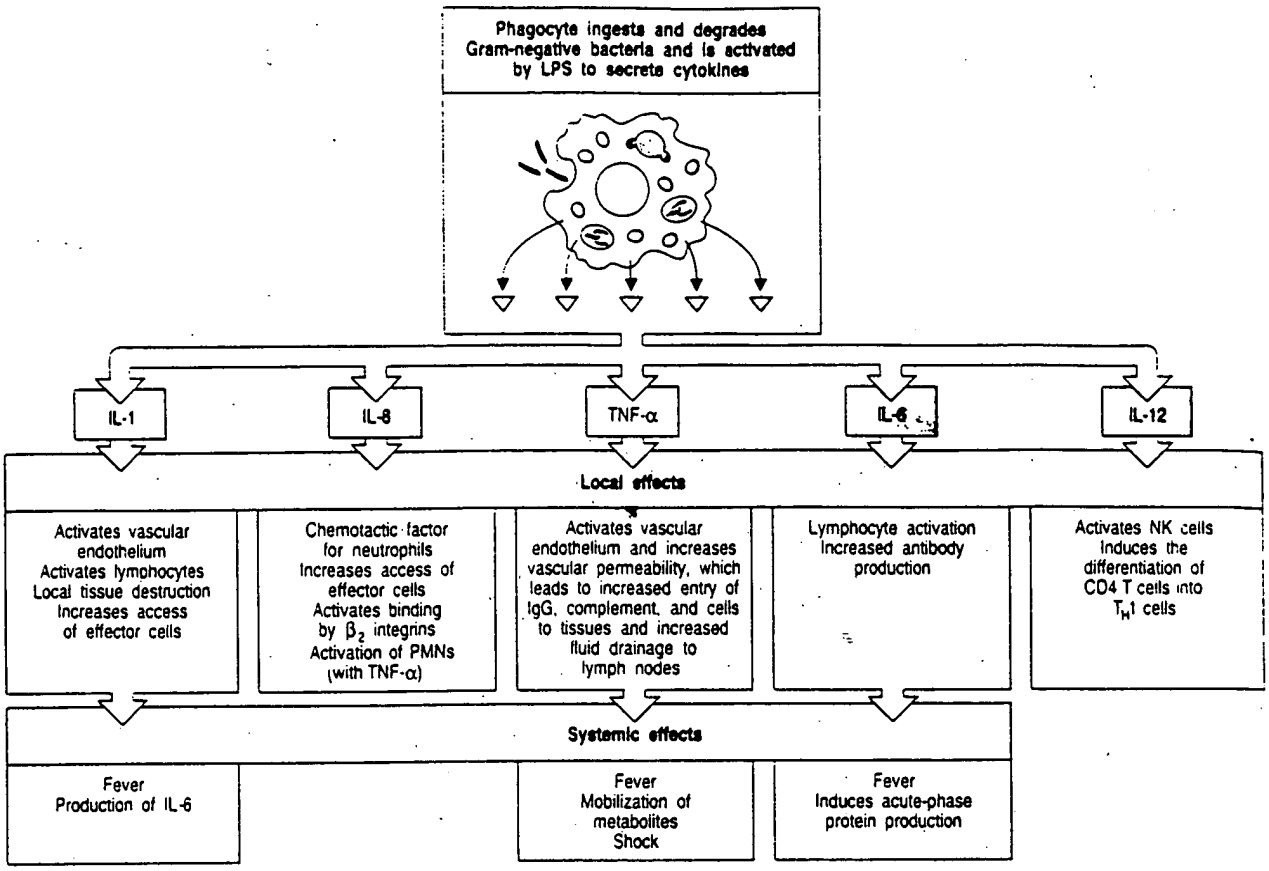


Fig. 10.10 Important cytokines secreted by macrophages in response to bacterial products include IL-1, IL-6, IL-8, IL-12 and TNF-α. TNF-α is an inducer of a local inflammatory response that helps to contain infections (see Section 10-8). It also has systemic effects, many of which are harmful. IL-1, IL-6, and TNF-α have a critical role in inducing the acute-phase response in the liver (see Section 10-11) and induce fever, which favors effective host defense in several ways. IL-8 is particularly important in directing neutrophil migration to sites of infection. IL-12 activates NK cells and favors the differentiation of T_H1 cells.

to release their granule contents, which include histamine, serotonin (in mice), and LTB4. These contribute to the changes in endothelial cells at sites of infection.

We have already discussed, in Section 9-20, the activation of mast cells and the actions of the inflammatory mediators that they release; however, when an individual first encounters a new pathogen there is unlikely to be any IgE of an appropriate specificity bound to the mast cells, so this route of activation is only likely to occur on re-infection. We shall return to the role of mast cells in inflammatory responses in Chapter 12, when we discuss allergic responses mediated by IgE.

The combined local effects of these mediators results in an **inflammatory response**, which is usually one of the immediate local reactions to infection. Inflammatory responses, which are operationally characterized by pain, redness, heat, and swelling at the site of an infection, reflect two types of change in the local blood vessels. The first of these is an increase in vascular diameter, leading to increased local blood flow—hence the heat and redness—and a reduction in the velocity of blood flow, especially along the surfaces of local blood vessels.

Under normal conditions, leukocytes are restricted to the center of blood vessels, where the flow is fastest. In inflammatory sites, where the vessels are dilated, the slower blood flow allows the leukocytes to move out of the center of the blood vessel and interact with the vascular endothelium. In addition to these changes, there is an increase in vascular permeability, leading to the local accumulation of fluid—hence the swelling and pain—as well as the accumulation of immunoglobulins, complement, and other blood proteins in the tissue.

The second effect of these mediators is to induce the expression of adhesion molecules on the endothelial cells of the local blood vessels, which bind to the surface of circulating monocytes and neutrophils and greatly increase the rate of migration of these phagocytic cells out of the blood and into the tissues. Even in the absence of infection, monocytes are migrating continuously into the tissues, where they differentiate into macrophages; during an inflammatory response, the induction of adhesion molecules on the endothelial cells, as well as induced changes in the adhesion molecules expressed on leukocytes, recruit large numbers of circulating leukocytes, initially neutrophils and later monocytes, into the site of an infection.

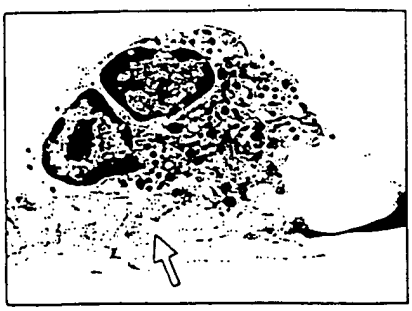
10-7 The migration of leukocytes out of blood vessels depends on adhesive interactions activated by the local release of inflammatory mediators.

The migration of leukocytes out of blood vessels, a process known as **extravasation**, is thought to occur in four steps. We shall describe this process as it is known to occur for monocytes and neutrophils (Fig. 10.11). Similar processes are thought to account for the homing of naive T lymphocytes to peripheral lymphoid organs and the delivery of effector T cells to sites of infection, as we shall see later.

The first step in this process involves selectins (see Fig. 8.5). The adhesive molecule P-selectin, which is carried inside endothelial cells in granules known as Weibel–Palade bodies, appears on endothelial cell surfaces within a few minutes of exposure to leukotriene B4, C5a, or histamine. A second selectin, E-selectin, appears a few hours after exposure to lipopolysaccharide or TNF-α. These selectins recognize carbohydrate epitopes, in this case the sialyl-Lewis^x moiety of certain leukocyte glycoproteins. The interaction of P-selectin and E-selectin with these surface glycoproteins allows monocytes and neutrophils to adhere reversibly to the vessel wall, so that circulating leukocytes can be seen to 'roll' along endothelium that has been treated with inflammatory cytokines (see Fig. 10.11, top panel). This adhesive interaction permits the stronger interactions of the second step in leukocyte migration.

The second step depends upon interactions between the leukocyte integrins known as LFA-1 (CD11a:CD18) and CR3 (CD11b:CD18—also called Mac-1) with molecules on endothelium such as the immunoglobulin-related adhesion molecule ICAM-1, which is also induced on endothelial cells by TNF-α (see Fig. 10.11, bottom panel). LFA-1 and CR3 normally adhere only weakly, but IL-8 or other chemoattractant cytokines (chemokines) trigger a conformational change in LFA-1 and CR3 on the rolling leukocyte, which greatly increases its adhesive capacity. In consequence, the leukocyte attaches firmly to the endothelium and the rolling is arrested.

In the third step, the leukocyte extravasates, or crosses the endothelial wall. This step also involves the leukocyte integrins LFA-1 and Mac-1, as well as a further adhesive interaction. This involves an immunoglobulin-related molecule called PECAM or CD31, which is expressed both on the leukocyte and



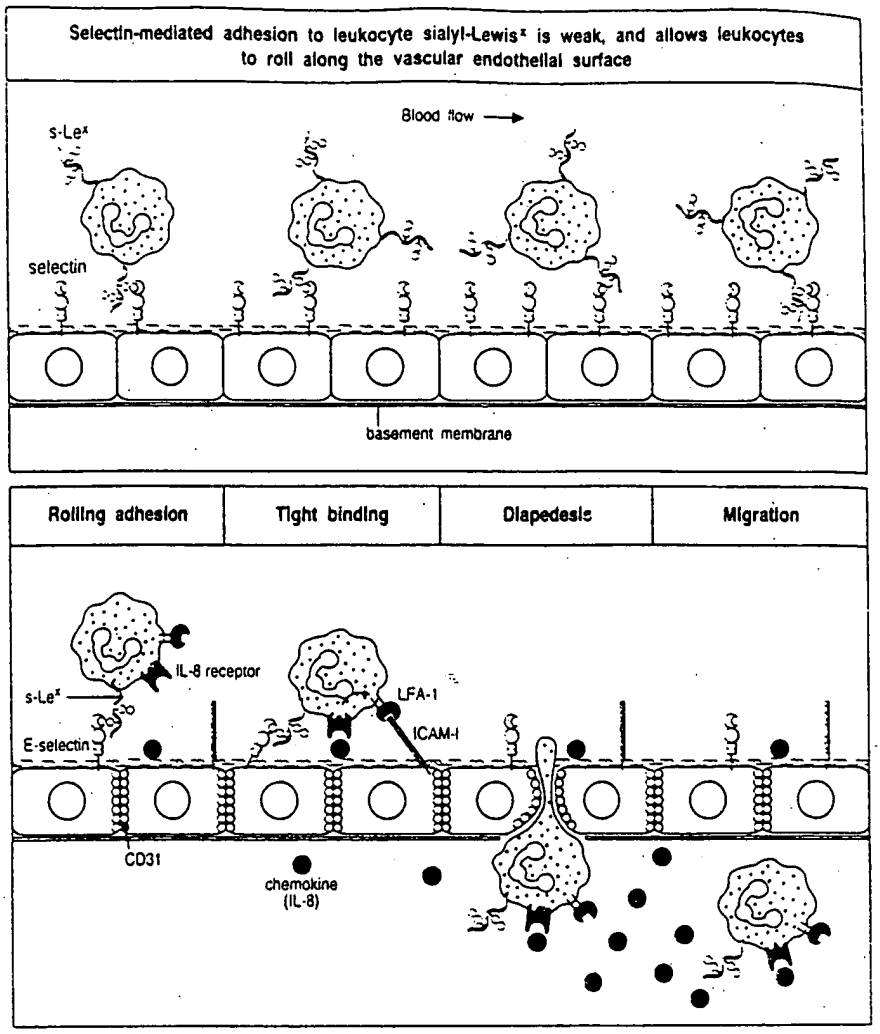


Fig. 10.11 Phagocytic leukocytes are directed to sites of infection through interactions between adhesion molecules induced by cytokines. The first step (top panel) involves the reversible binding of leukocytes to vascular endothelium through interactions between selectins induced on the endothelium and their carbohydrate ligands on the leukocyte, shown here for E-selectin and its ligand the sialyl-Lewis^x moiety (s-Le^x). This interaction cannot anchor the cells against the shearing force of the flow of blood and instead they roll along the endothelium, continually making and breaking contact. The binding does, however, allow stronger interactions, which occur as a result of the induction of ICAM-1 on the endothelium and the activation of its receptors LFA-1 and Mac-1 (not shown) on the leukocyte. Tight binding between these molecules arrests the rolling and allows the leukocyte to squeeze between the endothelial cells forming the wall of the blood vessel (ie to extravasate). The leukocyte integrins LFA-1 and Mac-1 are required for extravasation, and for migration toward chemoattractants. Adhesion between molecules of CD31, expressed on both the leukocyte and the junction of the endothelial cells, is also thought to contribute to diapedesis. The leukocyte also needs to traverse the basement membrane. Finally, the leukocyte migrates along a concentration gradient of chemokines (here shown as IL-8) secreted by cells at the site of infection. The electron micrograph shows a neutrophil extravasating between endothelial cells. Photograph (x 5500) courtesy of I Bird and J Spragg.

at the intercellular junctions of endothelial cells. These interactions enable the phagocyte to squeeze between the endothelial cells. It then penetrates the basement membrane (an extracellular matrix structure) with the aid of proteolytic enzymes that break down the proteins of the basement membrane. The movement through the vessel wall is known as **diapedesis**, and allows phagocytes to enter the site of infection. The fourth and final step in extravasation is the migration of the leukocytes through the tissues under the influence of chemokines. We shall discuss the activities of the small polypeptide cytokines known as chemokines in more detail later in this chapter.

10-8 TNF-α induces blood vessel occlusion and has an important role in containing local infection but can be fatal when released systemically.

The molecular changes induced at the endothelial cell surface by inflammatory mediators also induce the expression of molecules on endothelial cells that trigger blood clotting in the local small vessels, occluding them and cutting off blood flow. This can be important in preventing the pathogen from entering the bloodstream and spreading through the blood to organs all over the body. Instead, the fluid that has leaked into the tissue in the early phases of inflammation, carries the pathogen enclosed in phagocytic cells via the

lymph to the regional lymph nodes, where an adaptive immune response can be initiated. The importance of TNF-α in the containment of local infection is illustrated by experiments in which rabbits are infected locally with a bacterium. Normally, the infection will be contained at the site of the inoculation; if, however, an injection of anti-TNF-α antibody is also given, the infection spreads via the blood to other organs.

Once an infection spreads to the bloodstream, however, the same mechanisms whereby TNF-α so effectively contains local infection instead become catastrophic (Fig. 10.12). The presence of infection in the bloodstream,

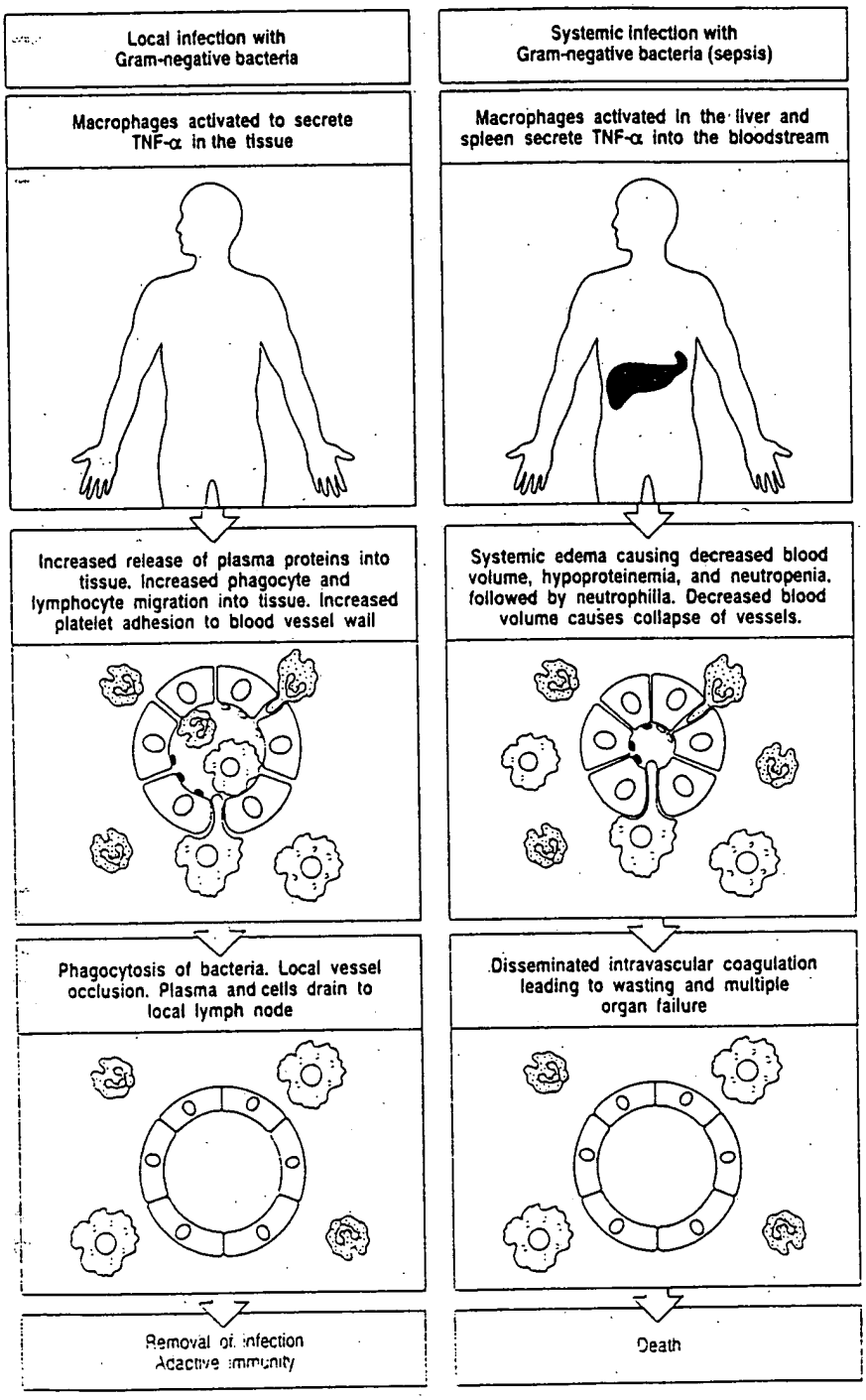


Fig. 10.12 The release of TNF-α by macrophages induces local protective effects, but TNF-α can have damaging effects when released systemically. The panels on the left show the causes and consequences of local release of TNF-α, while the panels on the right show the causes and consequences of systemic release. The central panels illustrate the common effects of TNF-α, which acts on blood vessels, especially venules, to increase blood flow, increase vascular permeability to fluid, proteins, and cells, and to increase endothelial adhesiveness for white blood cells and platelets. Local release thus allows an influx into the infected tissue of fluid, cells, and proteins that participate in host defense. The small vessels later clot, preventing spread of the infection to the blood, and the accumulated fluid and cells drain to regional lymph nodes where the adaptive immune response is initiated. When there is a systemic infection, or sepsis, with bacteria that elicit TNF-α production, then TNF-α is released into the blood and acts in a similar way on all small blood vessels. The result is shock, disseminated intravascular coagulation with depletion of clotting factors and consequent bleeding, multiple organ failure, and death.

known as sepsis, is accompanied by the release of TNF-α by macrophages in the liver, spleen, and other sites. The systemic release of TNF-α causes vasodilation and loss of plasma volume owing to increased vascular permeability, leading to shock. In septic shock, disseminated intravascular coagulation (blood clotting) is also triggered by TNF-α, leading to the generation of clots in the small vessels and the massive consumption of clotting proteins. The patient's ability to clot blood appropriately is lost. This condition frequently leads to the failure of vital organs such as the kidneys, liver, heart, and lungs, which are quickly compromised by the failure of normal perfusion; consequently, septic shock has a high mortality rate.

Mice with a mutant TNF-α receptor gene are resistant to septic shock; however, such mice are also unable to control local infection. Although the features of TNF-α that make it so valuable in containing local infection are precisely those that give it a central role in the pathogenesis of septic shock, it is clear from the evolutionary conservation of TNF-α that its benefits in the former arena outweigh the devastating consequences of its systemic release.

10-9 Small proteins called chemokines recruit new phagocytic cells to local sites of infection.

Some of the cytokines released in response to infection belong to a family of closely related proteins called **chemokines**, small polypeptides that are synthesized by phagocytes and by many other cell types. IL-8, whose contribution to extravasation we have just discussed, belongs to this subset of cytokines. All the chemokines are related in amino acid sequence and function mainly as chemoattractants for leukocytes, recruiting monocytes, neutrophils, and other effector cells from the blood to sites of infection. Some chemokines also function in lymphocyte development, migration, and angiogenesis (the growth of new blood vessels); the properties of some chemokines are listed in Fig. 10.13.

Members of the chemokine family fall mostly into two broad groups—CC chemokines with two adjacent cysteines, and CXC chemokines, in which the equivalent two cysteine residues are separated by another amino acid. The two groups of chemokines act on different sets of receptors and different cell types: in general, the CXC chemokines promote the migration of neutrophils, whereas the CC chemokines promote the migration of monocytes or other cell types. IL-8 is an example of a CXC chemokine; an example of a CC chemokine is the macrophage chemoattractant protein-1 (MCP-1). These two chemokines have similar, although complementary, functions: IL-8 induces neutrophils to leave the bloodstream and migrate into the surrounding tissues; MCP-1, in contrast, acts on monocytes, inducing their migration from the bloodstream to become tissue macrophages. Other CC chemokines such as RANTES may promote the infiltration into tissues of a range of leukocyte cell types including effector T cells (see Section 10-20), with individual chemokines acting on different subsets of cells. The only known C chemokine is called lymphotactin and is thought to attract T-cell precursors to the thymus. A newly discovered molecule called fractalkine is unusual in several ways: it has three amino acid residues between the two half-cysteines, making it a CX_3C chemokine; it is multimodular; and it is tethered to the membrane of cells that express it, where it serves both as a chemoattractant and as an adhesion protein.

The role of chemokines such as IL-8 and MCP-1 in cell recruitment is twofold: first, to convert the initial rolling of the leukocyte on the endothelial cells into stable binding; and second to direct its migration along a gradient of the

Class	Chemokine	Produced by	Receptors	Chemoattracted cells	Major effects
CXC	IL-8	Monocytes Macrophages Fibroblasts Keratinocytes Endothelial cells	CXCR1 CXCR2	Neutrophils Naive T cells	Mobilizes, activates and degranulates neutrophils Angiogenesis
	PBP β-TG NAP-2	Platelets	CXCR2	Neutrophils	Activates neutrophils Clot resorption Angiogenesis
	GROα, β, γ	Monocytes Fibroblasts Endothelium	CXCR2	Neutrophils Naive T cells Fibroblasts	Activates neutrophils Fibroplasia Angiogenesis
	IP-10	Keratinocytes Monocytes T cells Fibroblasts Endothelium	CXCR3	Resting T cells NK cells Monocytes	Immunostimulant Anti-angiogenic Promotes T_H1 immunity
	SDF-1	Stromal cells	CXCR4	Naive T cells Progenitor (CD34$^+$) B cells	B-cell development Lymphocyte homing Competes with HIV-1
CC	MIP-1α	Monocytes T cells Mast cells Fibroblasts	CCR1, 3, 5	Monocytes NK and T cells Basophils Dendritic cells	Competes with HIV-1 Anti-viral defense Promotes T_H1 immunity
	MIP-1β	Monocytes Macrophages Neutrophils Endothelium	CCR1, 3, 5	Monocytes NK and T cells Dendritic cells	Competes with HIV-1
	MCP-1	Monocytes Macrophages Fibroblasts Keratinocytes	CCR2B	Monocytes NK and T cells Basophils Dendritic cells	Activates macrophages Basophil histamine release Promotes T_H2 immunity
	RANTES	T cells Endothelium Platelets	CCR1, 3, 5	Monocytes NK and T cells Basophils Eosinophils Dendritic cells	Degranulates basophils Activates T cells Chronic inflammation
	Eotaxin	Endothelium Monocytes Epithelium T cells	CCR3	Eosinophils Monocytes T cells	Role in allergy
C	Lymphotactin	CD8>CD4 T cells	?	Thymocytes Dendritic cells NK cells	Lymphocyte trafficking and development
CXXXC (CX_3C)	Fractalkine	Monocytes Endothelium Microglial cells	CX_3CR1	Monocytes T cells	Leukocyte-endothelial adhesion Brain inflammation

Fig. 10.13 Properties of selected chemokines. Chemokines fall mainly into two related but distinct groups: the CC chemokines, which in humans are all encoded in one region of chromosome 4, have two adjacent cysteine residues; CXC chemokines, which are found in a cluster on chromosome 17, have an amino acid residue between the equivalent two cysteines. A C chemokine with only one cysteine at this location, and fractalkine, a CX_3C chemokine, are encoded elsewhere in the genome. Each chemokine interacts with one or more receptors, and affects one or more types of cell.

chemokine that increases in concentration towards the site of infection. This is achieved by the binding of the small, soluble chemokines to proteoglycan molecules in the extracellular matrix and on endothelial cell surfaces, thus displaying the chemokines on a solid substrate along which the leukocytes can migrate. Once the leukocytes have crossed the endothelium and the basement membrane to enter the tissues, their migration to the focus of infection is directed by the gradient of matrix-associated chemokine molecules.

Chemokines can be produced by a wide variety of cell types in response to bacterial products, viruses, and agents that cause physical damage, such as silica or the urate crystals that occur in gout. Thus, infection or physical damage to tissues sets in motion the recruitment of phagocytic cells to the site of damage. Both IL-8 and MCP-1 also activate their respective target cells, so that not only are neutrophils and macrophages brought to potential sites of infection but, in the process, they are armed to deal with any pathogens they may encounter. In particular, neutrophils exposed to IL-8 and TNF-α are activated to mediate a respiratory burst that generates oxygen radicals and nitric oxide, and to release their stored granule contents, thus contributing both to host defense and to local tissue destruction seen in local sites of infection with pyogenic (pus-forming) bacteria. Just as all the chemokines have similar structures, all their receptors are similar in structure; all are integral membrane proteins containing seven membrane-spanning helices. This structure is characteristic of receptors such as rhodopsin (see Fig. 8.32) and the muscarinic acetylcholine receptor, which are coupled to guanine nucleotide binding proteins (G-proteins); the chemokine receptors are also activated through coupled G-proteins.

Finally, there are several known examples of viruses that interfere with chemokine action and utilize chemokines or their receptors for their own ends. The most notorious of these is the human immunodeficiency virus-1 (HIV-1), the cause of AIDS. HIV-1 enters cells that express both CD4 and the chemokine receptor CCR5. People who are homozygous for a mutation in CCR5 are resistant to infection with HIV-1. We shall return to this in Chapter 11, where we discuss means adopted by pathogens to frustrate the immune response.

Thus, tissue phagocytes initiate host responses in tissues, and their numbers are soon augmented through the action of chemokines, which recruit large numbers of circulating phagocytic and immunocompetent cells to sites of infection and tissue damage. Why there are so many chemokines is not yet known; neither is the exact role of each one in host defense and in pathological responses.

10-10 Neutrophils predominate in the early cellular infiltrate into inflammatory sites.

Neutrophils are abundant in the blood but are absent from normal tissues. They are short-lived, surviving only a few hours after leaving the bone marrow. The innate immune response produces a variety of factors that are chemotactic for neutrophils and they rapidly emigrate from the blood to enter sites of infection, where they are the earliest phagocytic cells to be recruited. Later, they are followed by monocytes, the precursors of macrophages. Once in an inflammatory site, the neutrophils are able to eliminate many pathogens by phagocytosis.

The role of neutrophils in the phagocytosis of antibody-coated pathogens was discussed in Chapter 9, and where the individual has had a previous encounter with the pathogen this is likely to be the dominant mechanism by which microorganisms are removed. However, neutrophils are able to phagocytose bacteria even in the absence of specific antibodies and can thus provide a protective response in the first encounter with a pathogen. Bacterial cell wall components can be bound directly by several receptors on neutrophils. Neutrophils can also phagocytose microorganisms coated with the complement component C3b and its inactive derivative iC3b, which are deposited on the surface of the pathogen by the alternative pathway of complement activation (see Sections 9-26 and 10-4).

Neutrophils produce several bacteriostatic and toxic products, and phagocytosed pathogens are killed rapidly (see Section 9-18). Neutrophil granules containing enzymes, proteins, and peptides fuse with pathogen-containing phagosomes upon neutrophil activation, releasing these antibacterial agents on to the pathogen. The combination of toxic oxygen metabolites, nitric oxide, proteases, phospholipases, and antibacterial proteins and peptides is able to eliminate Gram-positive and Gram-negative bacteria, fungi, and even some enveloped viruses. The importance of neutrophils in host defense is best illustrated by considering inherited defects in neutrophil maturation or antibacterial functions; patients with such deficiencies suffer recurrent infections, often of bacteria and fungi that form part of the normal flora. In patients with no neutrophils such infections frequently escape from the local site to produce a life-threatening septicemia (infection of the blood). Neutrophils themselves are short-lived and the pus formed at sites of inflammation contains many dead and dying neutrophils. Any microorganisms that have been phagocytosed but not killed are released at this point and can be re-phagocytosed by other neutrophils or by macrophages that accumulate later in the inflammatory response. Even this sequestration of microorganisms can be important in host defense; in individuals whose neutrophils are unable to kill phagocytosed organisms, in contrast to those with no neutrophils at all, infections only rarely spread beyond the local inflammatory site.

10-11 Cytokines released by phagocytes also activate the acute-phase response.

As well as their important local effects, the cytokines produced by macrophages and neutrophils have long-range effects that contribute to host defense. One of these is the elevation of body temperature, which is caused by TNF-α, IL-1, IL-6, and other cytokines. These are termed 'endogenous pyrogens' because they cause fever and derive from an endogenous source rather than from bacterial components. Fever is generally beneficial to host defense; most pathogens grow better at lower temperatures and adaptive immune responses are more intense at raised temperatures. Host cells are also protected from deleterious effects of TNF-α at raised temperatures.

The effects of TNF-α, IL-1, and IL-6 are summarized in Fig. 10.14. One of the most important of these is the initiation of a response known as the acute-phase response (Fig. 10.15). This involves a shift in the proteins secreted by the liver into the blood plasma and results from the action of IL-1, IL-6,

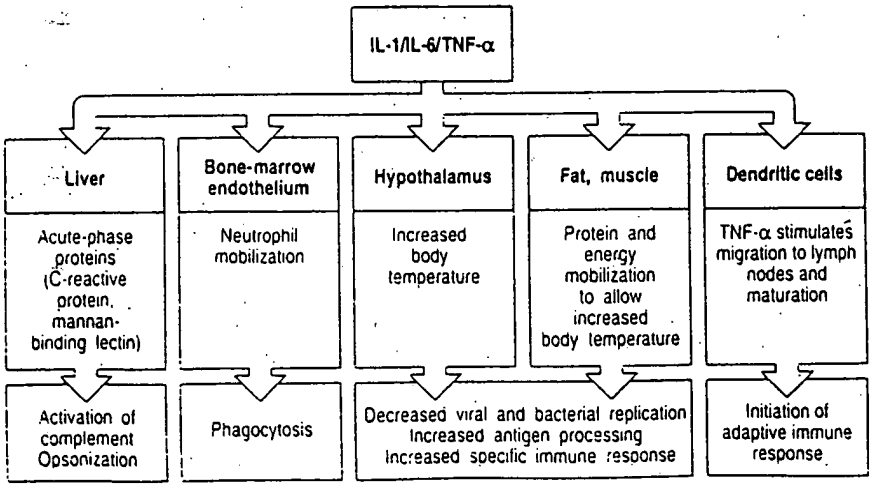


Fig. 10.14 The cytokines TNF-α, IL-1, and IL-6 have a wide spectrum of biological activities that help to co-ordinate the body's responses to infection. IL-1, IL-6, and TNF-α activate hepatocytes to synthesize acute-phase proteins, and bone-marrow endothelium to release neutrophils. The acute-phase proteins act as opsonins, whereas the disposal of opsonized pathogens is augmented by enhanced recruitment of neutrophils from the bone marrow. IL-1, IL-6, and TNF-α are also endogenous pyrogens, raising body temperature, which is believed to help to eliminate infections. A major effect of these cytokines is to act on the hypothalmus, altering the body's temperature regulation, and on muscle and fat cells, altering energy mobilization to increase the body temperature. At elevated temperatures, bacterial and viral replication are decreased, whereas processing of antigen is enhanced. Finally, they help activate B and T cells by inducing migration to lymph nodes and maturation of dendritic cells to induce an adaptive immune response.

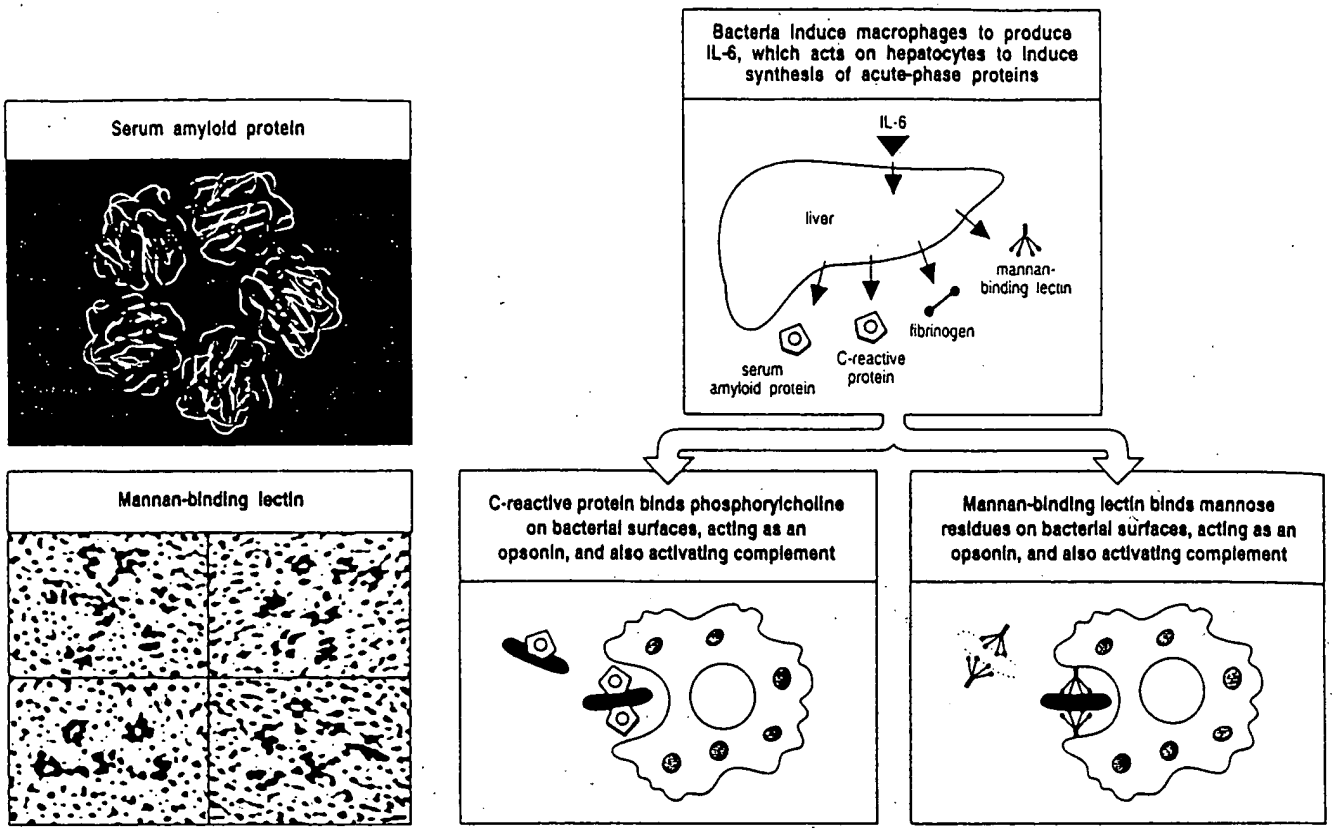


Fig. 10.15 The acute-phase response produces molecules that bind bacteria but not host cells. Acute-phase proteins are produced by liver cells in response to cytokines released by phagocytes in the presence of bacteria. They include serum amyloid protein (SAP) (in mice but not humans), C-reactive protein (CRP), fibrinogen, and mannan-binding lectin (MBL). SAP and CRP are homologous in structure; both are pentraxins, forming five-membered disks, as shown for SAP (upper photograph). CRP binds phosphorylcholine on bacterial surfaces but does not recognize it in the form in which it is found in host-cell membranes, and can both act as an opsonin in its own right and activate the classical complement pathway by binding C1q to augment opsonization. MBL is a member of the collectin family, which includes C1q, which it resembles in its structure (see lower photograph and Fig. 9.34). MBL binds mannose residues on bacterial cell surfaces and, like CRP, can both act as an opsonin in its own right and activate complement. MBL activates the lectin complement pathway by binding and activating two serine esterases, resembling C1rs, that in turn activates C4 and C2. Thus, CRP and MBL can lead to bacterial clearance in the same way as an IgM antibody. Photographs courtesy of J Emsley (SAP) and K Reid (MBL).

and TNF-α on hepatocytes. In the acute-phase response, levels of some plasma proteins go down, while levels of others increase markedly. The proteins whose synthesis is induced by TNF-α, IL-1, and IL-6 are called acute-phase proteins. Of the acute-phase proteins, two are of particular interest because they mimic the action of antibodies but, unlike antibodies, these proteins have broad specificity for pathogen molecules.

One of these proteins, C-reactive protein, is a member of the pentraxin protein family, so called because they are formed from five identical subunits. C-reactive protein binds to the phosphorylcholine portion of certain bacterial and fungal cell wall lipopolysaccharides. Phosphorylcholine is also found in mammalian cell membrane phospholipids but in a form that cannot react with C-reactive protein. When C-reactive protein binds to a bacterium, it is not only able to opsonize it but can also activate the complement cascade by binding to C1q, the first component of the classical pathway of complement activation. The interaction with C1q involves the collagen-like parts of C1q, rather than the globular heads contacted by antibody, but the same cascade of reactions is initiated.

The second acute-phase protein of interest is **mannan-binding lectin** (MBL). This is found in normal serum at low levels but is also produced in increased amounts during the acute-phase response. It is a calcium-dependent sugar-binding protein, or lectin, a member of a structurally related family of proteins known as the **collectins**. It binds to mannose residues, which are accessible on many bacteria but are covered by other sugar groups in the carbohydrates on vertebrate cells. MBL also acts as an opsonin for monocytes, which, unlike tissue macrophages, do not express the macrophage mannose receptor. The structure of mannan-binding lectin resembles that of the C1q component of complement, although the two proteins do not share sequence homology. When it binds to bacteria, mannan-binding lectin can, like C1q, activate a proteolytic enzyme complex that cleaves C4 and C2 to initiate complement activation by the lectin pathway (see Fig. 10.8). The collectins also include the pulmonary surfactant proteins A and D (SP-A and SP-D), which are probably important in binding and opsonizing pulmonary pathogens such as *Pneumocystis carinii*.

Thus, within a day or two, the acute-phase response provides the host with two proteins with the functional properties of antibodies and which can bind a broad range of bacteria. However, unlike antibodies, they have no structural diversity, and are made in response to any stimulus that triggers the release of TNF-α, IL-1, and IL-6, so their synthesis is not specifically induced and targeted.

A final distant effect of the cytokines produced by phagocytes is to induce a leukocytosis, an increase in circulating neutrophils. The leukocytes come from two sources: the bone marrow, from which mature leukocytes are released in increased numbers; and sites in blood vessels where the leukocytes are attached loosely to endothelial cells. Finally, the migration of dendritic cells from their sites in peripheral tissues to the lymph node, and their maturation into non-phagocytic but highly co-stimulatory antigen-presenting cells is stimulated by TNF-α. Such cells are crucial to the initiation of adaptive immunity, as we shall see in Section 10-16. All these effects of cytokines produced in response to infection contribute to the control of infection while the adaptive immune response is being developed.

10-12 Interferons inhibit viral replication and activate certain host-defense responses.

Infection of cells with viruses induces the production of proteins known as interferons because they were found to interfere with viral replication in previously uninfected tissue culture cells. They are believed to have a similar role *in vivo*, blocking the spread of viruses to uninfected cells. These antiviral interferons, called **interferon-α** (IFN-α) and **interferon-β** (IFN-β), are quite distinct from **interferon-γ** (IFN-γ), which is produced by activated NK cells and in larger amounts by effector T cells and thus appears mainly after the induction of the adaptive immune response. IFN-α, actually a family of several closely related proteins, and IFN-β, the product of a single gene, are synthesized by many cell types after viral infection. Double-stranded RNA is a potent inducer of interferon synthesis. It is not found in mammalian cells but forms the genome of some viruses and might be made as part of the infectious cycle of all viruses; it might be the common element in interferon induction. Interferons make several contributions to host defense against viral infection (Fig. 10.16).

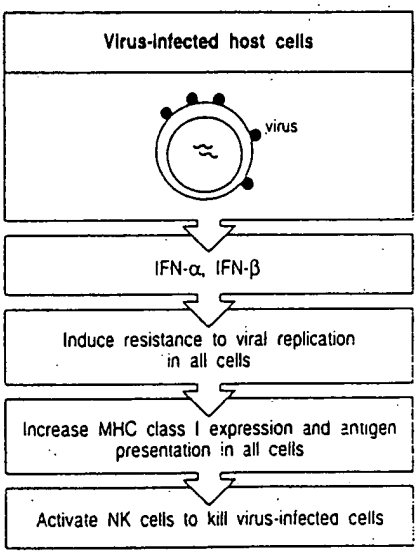


Fig. 10.16 Interferons are antiviral proteins produced by cells in response to viral infection. The α- and β-interferons have three major functions. First, they induce resistance to viral replication by activating cellular genes that destroy mRNA and inhibit the translation of viral and some host proteins. Second, they induce MHC class I expression in most uninfected cells in the body, thus enhancing the resistance to natural killer (NK) cells, and make cells newly infected by virus more susceptible to killing by CD8 cytotoxic T cells. Third, they activate NK cells, which then kill virus-infected cells selectively.

An obvious and important effect of interferons is the induction of a state of resistance to viral replication in all cells. IFN-α and IFN-β bind to a common cellular receptor on cells. The interferon receptor, like other cytokine receptors (see Chapter 8), is coupled to a Janus-family tyrosine kinase, which in turn phosphorylates signal-transducing activators of transcription known as STATs. The binding of phosphorylated STAT proteins to the promoters of several genes induces the synthesis of host-cell proteins that contribute to the inhibition of viral replication. One of these is the enzyme oligo-adenylate synthetase, which polymerizes ATP into a series of 2′–5′ linked oligomers (nucleotides in nucleic acids are normally linked 3′–5′). These activate an endoribonuclease that then degrades viral RNA. A second protein activated by IFN-α and IFN-β is a serine/threonine kinase called P1 kinase. This enzyme phosphorylates the eukaryotic protein synthesis initiation factor eIF-2, thereby inhibiting translation and thus contributing to the inhibition of viral replication. Another interferon-inducible protein called Mx is known to be required for cellular resistance to influenza virus replication. Mice that lack the gene for Mx are highly susceptible to infection with the influenza virus, whereas genetically normal mice are not.

The second effect of interferons in host defense is to increase the expression of MHC class I molecules, TAP transporter proteins, and the Lmp2 and Lmp7 components of the proteasome. This enhances the ability of host cells to present viral peptides to CD8 T cells should infection occur (see Section 4-10). At the same time, this increase in MHC class I expression protects uninfected host cells against attack by natural killer cells (NK cells). Natural killer cells are strongly activated by IFN-α and IFN-β, and make several important contributions to early host responses to viral infections, as we shall see next.

10-13 Natural killer cells serve as an early defense against certain intracellular infections.

Natural killer, or NK cells, which we introduced in Chapter 9 as the effectors in antibody-dependent cell-mediated cytotoxicity, are identified by their ability to kill certain lymphoid tumor cell lines *in vitro* without the need for prior immunization or activation. However, their known function in host defense is in the early phases of infection with several intracellular pathogens, particularly herpes group viruses, *Leishmania*, and *Listeria monocytogenes*, and we shall consider them here from that point of view.

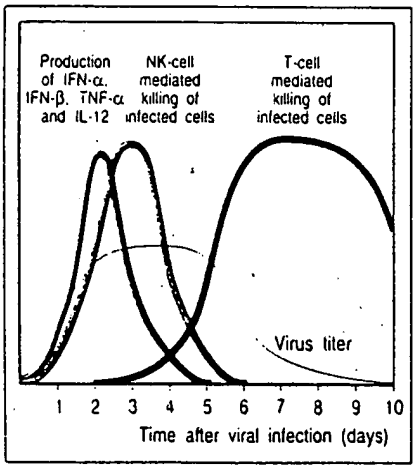


Fig. 10.17 Natural killer cells (NK cells) are an early component of the host response to virus infection. Interferons-α and -β and the cytokines TNF-α and IL-12 appear first, followed by a wave of NK cells, which together control virus replication but do not eliminate the virus. Virus elimination is accomplished when specific CD8 T cells are produced. Without NK cells, the levels of certain viruses are much higher in the early days of the infection.

Although NK cells that can kill sensitive targets can be isolated from uninfected individuals, this activity is increased by between 20- and 100-fold when NK cells are exposed to IFN-α and IFN-β or to the NK-cell activating factor IL-12, which is one of the cytokines produced early in many infections (Fig. 10.17). IL-12, in synergy with TNF-α, can also elicit the production of large amounts of IFN-γ by NK cells, and this secreted IFN-γ is crucial in controlling some infections before T cells have been activated to produce this cytokine. One example is the response to the intracellular bacterium *Listeria monocytogenes*. Mice that lack T and B lymphocytes are initially quite resistant to this pathogen; however, antibody-mediated depletion of NK cells or neutralization of TNF-α or IFN-γ or their receptors renders these mice highly susceptible, so that they die a few days after infection.

If NK cells are to mediate host defense against infection with viruses and other pathogens, they must have some mechanism for distinguishing infected from uninfected cells. Exactly how this is achieved has not yet been worked out, but recognition of 'altered self' is thought to be involved. NK cells have

two types of surface receptor that control their cytotoxic activity. One type triggers killing by NK cells; several receptors can provide this activation signal, including calcium-binding C-type lectins that recognize a wide variety of carbohydrate ligands found on many cells. A second set of receptors inhibit activation, and prevent NK cells from killing normal cells. These inhibitory receptors are specific for MHC class I alleles, which explains why NK cells selectively kill target cells bearing low levels of MHC class I molecules. Thus, one possible mechanism by which NK cells distinguish infected from uninfected cells is by recognizing alterations in MHC class I expression (Fig. 10.18). Another is that they recognize changes in cell-surface glycoproteins induced by viral or bacterial infection.

In mice, receptors that inhibit the activation of NK cells are encoded by a multigene family of C-type lectins called Ly49. Different Ly49 receptors recognize different MHC class I alleles and are differentially expressed on different subsets of NK cells. Some NK cells express Ly49 receptors specific for non-self MHC alleles, but each cell expresses at least one receptor that can recognize an MHC class I allele expressed by the host. In humans, there are inhibitory receptors that recognize distinct HLA-B and HLA-C alleles. These receptors are structurally different from those of the mouse, being members of the immunoglobulin gene superfamily; they are usually called p58 and p70, or killer inhibitory receptors (KIRs). In addition, human NK cells express a heterodimer of two C-type lectin molecules, called CD94 and NKG2. Other

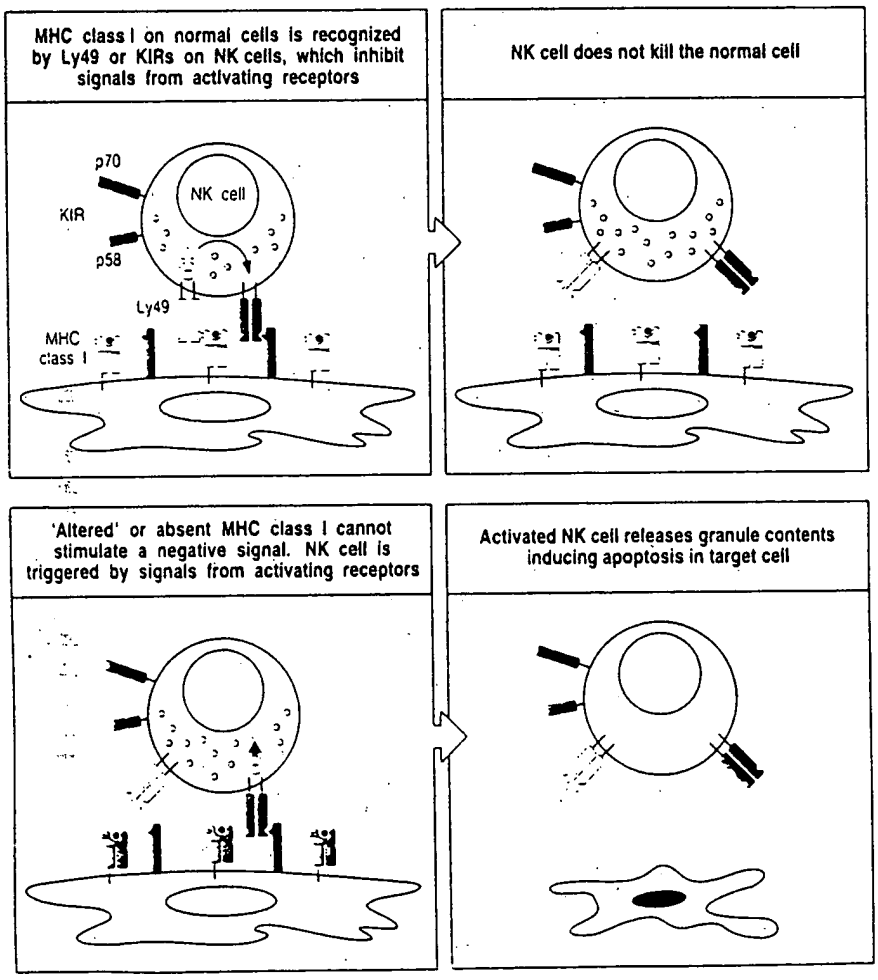


Fig. 10.18 Possible mechanisms whereby NK cells distinguish infected from non-infected cells. A proposed mechanism of NK cell recognition is shown. NK cells can use several different receptors that signal them to kill, including lectin-like receptors that recognize carbohydrate on self cells. However, another set of receptors, called Ly49 in the mouse and killer inhibitory receptors (KIR) in the human, recognize MHC class I molecules and inhibit killing by NK cells by overruling the actions of the killer receptors. This inhibitory signal is lost when host cells do not express MHC class I and perhaps also in cells infected with virus, which might inhibit MHC class I expression or alter its conformation. Normal cells respond to IFN-α and -β by increasing levels of MHC class I expression, making them resistant to activated NK killing. Infected cells can fail to increase MHC class I expression, making them targets for activated NK cells. Ly49 and KIR are encoded by different families of genes—the C-type lectins for Ly49 and the immunoglobulin gene superfamily for the KIRs. The KIRs are made in two forms, p58 and p70, which differ by the presence of one immunoglobulin domain.

inhibitory NK receptors specific for the products of the MHC class I loci are being defined at a rapid rate, and all are members of either the immunoglobulin-like KIR family or the Ly49-like C-type lectin family. A common feature of all inhibitory NK receptors is the presence of an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains (see Fig. 5.19).

Because the binding of the inhibitory receptors to MHC class I molecules inhibits NK activity, normal syngeneic cells are protected from attack by NK cells. Virus-infected cells, however, can become susceptible to killing by NK cells by a variety of mechanisms. First, some viruses inhibit all protein synthesis in their host cells, so the augmented synthesis of MHC class I proteins induced by interferon would be blocked selectively in infected cells, and NK cells would no longer be inhibited through their MHC-specific receptors. Second, some viruses can selectively prevent the export of MHC class I molecules, which might allow the infected cell to evade recognition by CD8 T cells but would make it sensitive to NK cell killing. There is also evidence that introduction of new peptides into self MHC class I molecules is detectable by NK cells. It is not known whether these peptides are recognized directly or whether they alter MHC conformation. Finally, virus infection alters the glycosylation of cellular proteins, perhaps allowing dominant recognition by activation receptors or removing the normal ligand for the inhibitory receptors. Either of these last two mechanisms could allow infected cells to be detected even when the level of MHC class I expression has not been altered.

Clearly, much remains to be learned about this innate mechanism of cytotoxic attack and its physiological significance. At present, the only clue to the function of NK cells in humans comes from a rare patient deficient in NK cells who proved highly susceptible to early phases of herpes virus infection. The ability of NK cells to operate early in host defense by mechanisms that involve the recognition of self MHC molecules suggests they might represent the modern remnants of the evolutionary forebears of T cells. Two other 'primitive' lymphocyte types—γ:δ T cells and B-1 cells—might also participate in the pre-adaptive immune response. Of these, the γ:δ T cells, which we discuss first, are the more enigmatic.

10-14 T cells bearing γ:δ T-cell receptors are found in lymphoid organs and most epithelia and might contribute to host defense by regulating the behavior of other cells.

The discovery of γ:δ T cells was accidental; they were detected as a consequence of having immunoglobulin-like receptors encoded by rearranged genes (see Section 4-30), and their function remains obscure. One of their most striking features is their division into two highly distinct sets of cells. One set of γ:δ T cells is found in the lymphoid tissue of all vertebrates and, like B cells and α:β T cells, they display highly diverse receptors. By contrast, intra-epithelial γ:δ T cells occur variably in different vertebrates, and commonly display receptors of very limited diversity, particularly in the skin and the female reproductive tract of mice, where the γ:δ cells are essentially homogeneous in any one site. On the basis of this limited diversity of epithelial γ:δ T cells and their limited recirculatory behavior, it has been proposed that intra-epithelial γ:δ T cells recognize ligands that are derived from the epithelium in which they reside and signify that it has become infected. Candidate ligands are heat-shock proteins, MHC class IB molecules, and unorthodox nucleotides and phospholipids, for all of which there is evidence of recognition by γ:δ T cells. Unlike α:β T cells, γ:δ T cells do not generally recognize antigen as peptides presented by MHC molecules; instead they seem to recognize their

target antigens directly, and could potentially recognize and respond rapidly to molecules expressed by many different cell types. Recognition of molecules expressed as a consequence of infection, rather than of pathogen-specific antigens themselves, would distinguish γδ T cells from other lymphocytes and arguably place them at the intersection between innate and adaptive immunity.

Several recent studies of mice deficient in γδ T cells have revealed exaggerated responses to various pathogens and even to self tissues, rather than deficiencies in pathogen control and rejection. This has led to the suggestion that at least some γδ T cells have a regulatory role in modulating immune responses, a function that would be consistent with their demonstrated ability to secrete regulatory cytokines when activated. Which aspects of the phenotype of γδ-deficient mice are attributable to which subset of γδ T cells remains to be clarified.

10-15 B-1 cells form a separate population of B cells, producing antibodies against common bacterial polysaccharides.

The production of antibody by conventional B cells has a major role in the adaptive immune response. However, there is a separate lineage of B cells, marked by the cell-surface protein CD5, that have properties quite distinct from those of conventional B cells (see Section 6-13). These so-called CD5 B cells, or B-1 cells, are in many ways analogous to epithelial γδ T cells: they arise early in ontogeny, they use a distinctive and limited set of V genes to make their receptors, they are self-renewing in the periphery, and they are the predominant lymphocyte in a distinctive microenvironment, the peritoneal cavity.

B-1 cells seem to make antibody responses mainly to polysaccharide antigens of the TI-2 type. These T-cell independent responses do not induce significant class switching or somatic hypermutation of immunoglobulin variable regions; as a consequence, the predominant antibody isotype produced is IgM (Fig. 10.19). Although these responses can be augmented by T cells, with IL-5 having an important role (see Section 9-10), they appear within 48 hours of the exposure to antigen, and the T cells involved are therefore not part of an antigen-specific adaptive immune response. The lack of an antigen-specific interaction with helper T cells might explain why immunological memory is not generated: repeated exposures to the same TI-2 antigen elicit similar or decreased responses with each exposure to antigen. Thus, these responses, although generated by lymphocytes with rearranging receptors, resemble innate rather than adaptive immune responses.

As with γδ T cells, the precise role of B-1 cells in host defense is uncertain. Mice that are deficient in B-1 cells are more susceptible to infection with *Streptococcus pneumoniae*: this is because they fail to produce an antibody against the phospholipid headgroup phosphorylcholine that effectively protects against this organism. The phosphorylcholine is bound to carbohydrate in the bacterial cell wall and is recognized as a TI-2 antigen because it can very effectively crosslink the B-1 cell antigen receptor. A significant fraction of the B-1 cells can make antibodies of this specificity, and because no antigen-specific T-cell help is required, a potent response can be produced early in infection with this pathogen. Whether human B-1 cells have the same role is uncertain. In terms of evolution, it is interesting to note that γδ T cells seem to defend the body surfaces, whereas B-1 cells defend the body cavity. Both cell types are relatively limited in their range of specificities and in the efficiency of

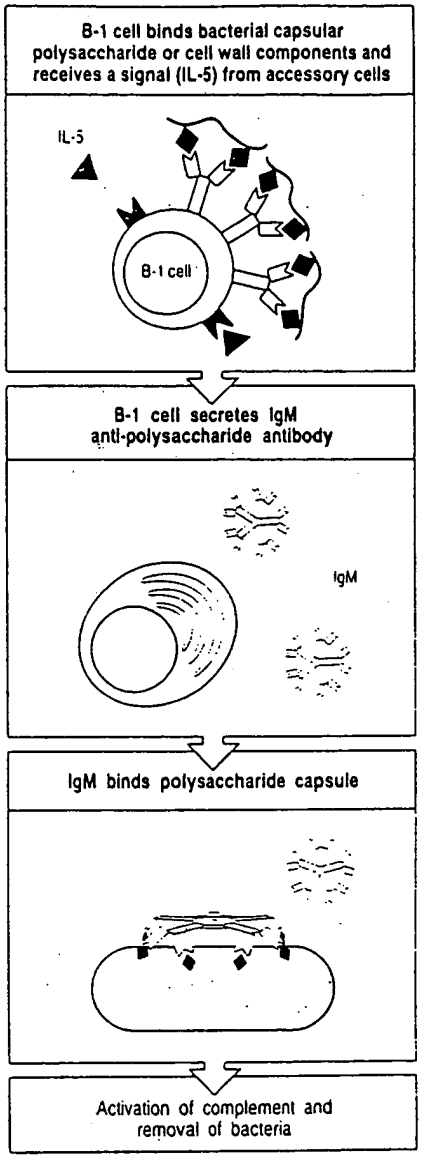


Fig. 10.19 CD5 B cells might be important in the response to carbohydrate antigens such as bacterial polysaccharides. These TI-2 responses might require IL-5 provided by T cells but this help is not antigen specific and its mechanism is not clear. These responses are rapid, with antibody appearing in 48 hours, presumably because there is a high frequency of precursors of the responding lymphocytes so that little clonal expansion is required. In the absence of antigen-specific T-cell help, only IgM is produced and, in mice, these responses therefore work mainly through the activation of complement.

their responses. It is possible that these two cell types represent a transitional phase in the evolution of the adaptive immune response, guarding the two main compartments of primitive organisms—the epithelial surfaces and the body cavity. It is not yet clear whether they are still critical to host defense or whether they represent an evolutionary relic. Nevertheless, as each cell type is prominent in certain sites in the body and contributes to certain responses, they must be incorporated into our thinking about host defense.

Summary.

The early induced but non-adaptive responses to infection involve a wide variety of effector mechanisms directed at distinct classes of pathogen. These responses are triggered by receptors that are either non-clonal or of very limited diversity, and are distinguished from adaptive immunity by their failure to provide lasting immunity or immunological memory. Some are induced by cytokines released by phagocytes in response to microbial infection. These cytokines have three major effects. First, they induce the production of acute-phase proteins by the liver, which can bind to bacterial surface molecules and activate complement or phagocytes. Second, they can elevate body temperature, which is thought to be deleterious to the microorganism but to enhance the immune response. Third, they induce inflammation, in which the surface properties and permeability of blood vessels are changed, recruiting phagocytes, immune cells, and molecules to the site of infection. Interferons are produced by cells infected with viruses, and these slow viral replication and enhance the presentation of viral peptides to cytotoxic T cells, as well as activating natural killer cells (NK cells), which can distinguish infected from uninfected host cells. NK cells, B-1 cells, and γδ T cells are lymphocytes with receptors of limited diversity that seem to provide early protection from a limited range of pathogens but do not generate lasting immunity or immunological memory. All these mechanisms have an important role, both on their own in holding infection in check during its early phases while the adaptive immune response is being developed, and also in their impact on the adaptive immune response that develops subsequently.

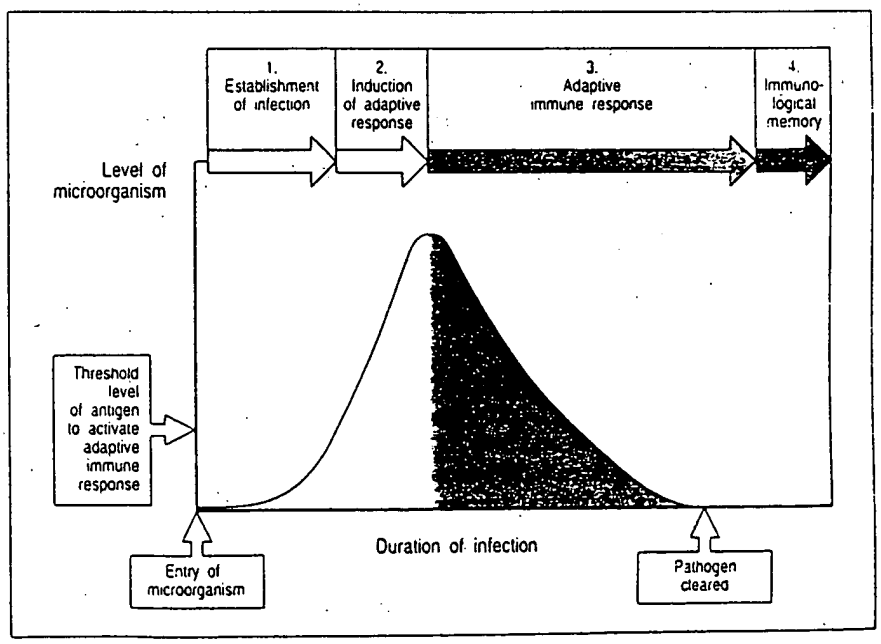


Fig. 10.20 The course of a typical acute infection. 1. The level of infectious agent increases with pathogen replication. 2. When the pathogen level exceeds the threshold dose of antigen required for an adaptive response, the response is initiated; the pathogen continues to grow, retarded only by the innate and early, non-adaptive responses. 3. After 4–5 days, effector cells and molecules of the adaptive response start to clear the infection. 4. When the infection is cleared, and the dose of antigen falls below the response threshold, the response ceases but antibody, residual effector cells, and also immunological memory provide lasting protection against re-infection.

Adaptive immunity to infection.

It is not known how many infections are dealt with solely by non-adaptive mechanisms of host defense; this is because they are eliminated early and such infections produce little in the way of symptoms or pathology. Moreover, deficiencies in non-adaptive defenses are rare, so it has seldom been possible to study their consequences. Adaptive immunity is triggered when an infection eludes the innate defense mechanisms and generates a threshold dose of antigen (Fig. 10.20). This antigen then initiates an adaptive immune response, which becomes effective only after several days, the time required for antigen-specific T and B cells to proliferate and differentiate into effector cells. Meanwhile, the pathogen continues to grow in the host, held in check mainly by innate and non-adaptive mechanisms (Fig. 10.21). In the earlier chapters of this book we discussed the cells and molecules that mediate the adaptive immune response, and the interactions between cells that stimulate individual steps in its development. We are now ready to see how each cell type is recruited in turn in the course of a primary immune response to a pathogen, and how the effector cells and molecules that are generated in response to antigen are dispersed to their sites of action, leading to clearance of the infection and the establishment of a state of protective immunity.

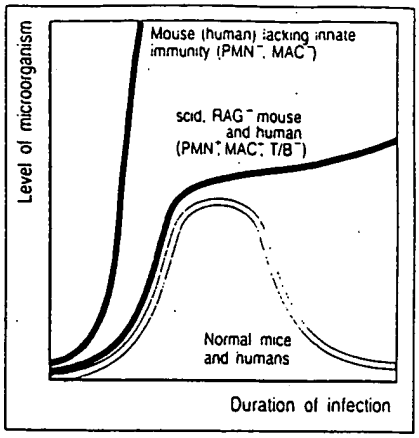


Fig. 10.21 The time course of infections in various types of immunodeficient mice and humans. The red curve shows the growth of microorganisms in the absence of innate immunity. The green curve indicates mice and humans that have innate immunity but lack adaptive immunity. The yellow curve shows the normal course of an infection in immunocompetent mice or humans. PMN, polymorphonuclear leukocytes; MAC, macrophages; T, T cells; B, B cells.

10-16 T-cell activation is initiated when recirculating T cells encounter specific antigen in draining lymphoid tissues.

The first step in any adaptive immune response leading to protective immunity is the activation of naive T cells in the draining lymphoid organs. The importance of the peripheral lymphoid organs was first shown by ingenious experiments in which a skin flap was isolated from the body wall so that it had a blood circulation but no lymphatic drainage. Antigen placed in this site did not elicit a T-cell response, showing that T cells do not become sensitized in peripheral tissues. We now know that naive T lymphocytes become sensitized in lymphoid organs by antigens taken up by dendritic cells. As noted in Chapter 8, immature dendritic cells in tissues are stimulated by infection to migrate to draining lymph nodes through which naive T cells circulate. Antigens introduced directly into the bloodstream are picked up by antigen-presenting cells in the spleen, and lymphoid cell sensitization then occurs in the splenic white pulp (see Fig. 1.9). The trapping of antigen by antigen-presenting cells that migrate to these lymphoid tissues, and the continuous recirculation of naive T cells through these tissues, ensure that rare antigen-specific T cells will encounter their specific antigen on a professional antigen-presenting cell surface.

The recirculation of naive T cells through the lymphoid organs is orchestrated by adhesive interactions between lymphocytes and endothelial cells. Naive T cells enter the lymphoid organs in essentially the same way as described earlier for the entry of phagocytes into sites of infection (see Fig. 10.11), except that in this case the selectin is expressed on the T cell rather than the endothelium. L-selectin on naive T cells binds to sulfated carbohydrates on various proteins such as the vascular addressins GlyCAM-1 and CD34. CD34 is expressed on endothelial cells in many tissues but is properly glycosylated for L-selectin binding only on the high endothelial venule cells of lymph nodes. L-selectin binding promotes a rolling interaction like that mediated by

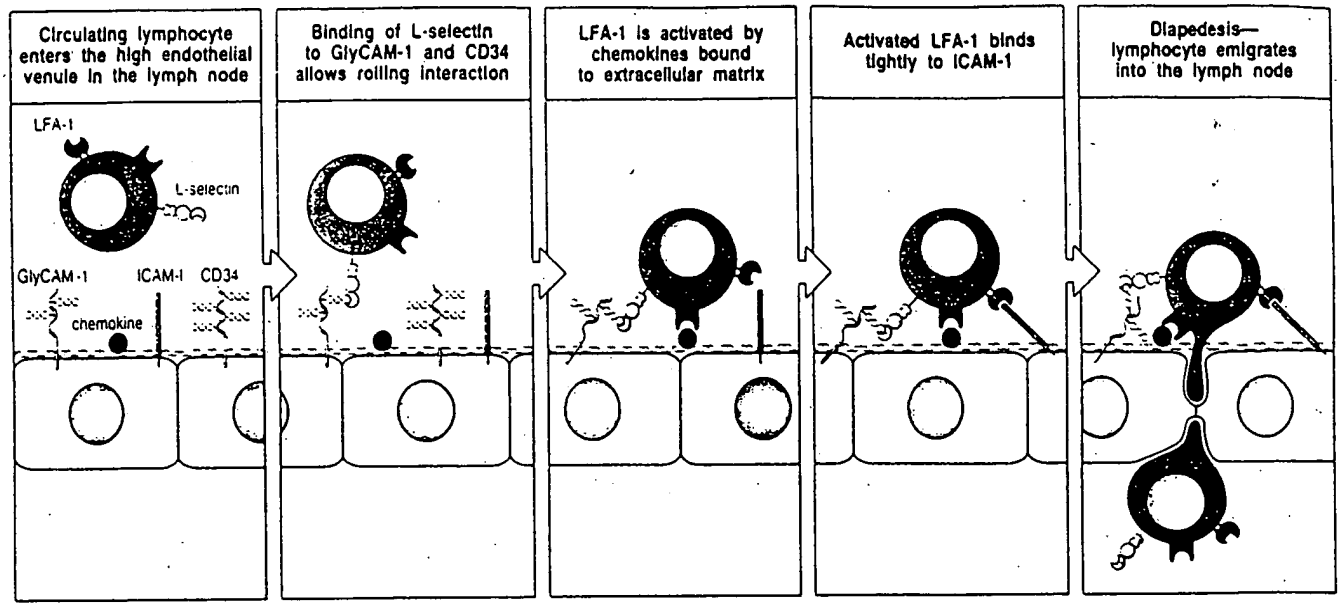


Fig. 10.22 Lymphocytes in the blood enter lymphoid tissue by crossing high endothelial venules. The first step in lymphocyte entry is the binding of L-selectin on the lymphocyte to sulfated carbohydrates of many proteins including GlyCAM-1 and CD34 on the high endothelial venule cell. Local chemokines activate LFA-1 on the lymphocyte and cause it to bind tightly to ICAM-1 on the endothelial cell, allowing transendothelial migration.

P- and E-selectin when they bind to the surface of phagocytes. This interaction is critical to the selectivity of naive lymphocyte homing. Although this interaction is too weak to promote extravasation, it is essential for the initiation of the stronger interactions that then follow between the T cell and the high endothelium, which are mediated by molecules with a relatively broad tissue distribution.

Stimulation by locally bound chemokines activates the adhesion molecule LFA-1 on the T cell, increasing its affinity for ICAM-2, which is expressed constitutively on all endothelial cells, and ICAM-1, which, in the absence of inflammation, is expressed only on the high endothelial venule cells of peripheral lymphoid tissues. The binding of LFA-1 to its ligands ICAM-1 and ICAM-2 has a major role in T-cell adhesion to and migration through the wall of the blood vessel into the lymph node (Fig. 10.22).

The high endothelial venules are located in the T-cell rich zone of the lymph nodes. This area is also inhabited by dendritic cells that have recently migrated from nearby tissues and developed potent co-stimulatory capacity. The migrating T cells scan the surface of these dendritic cells for specific peptide:MHC complexes. If they do not recognize antigen presented by these

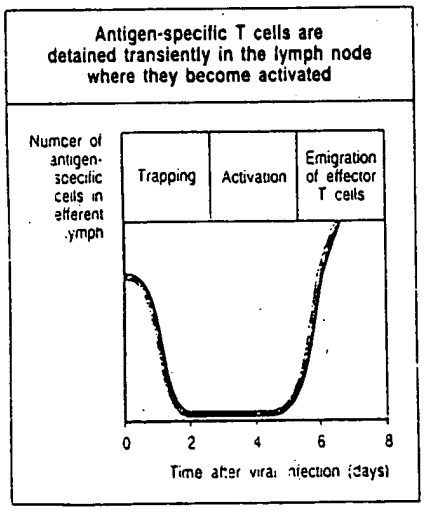


Fig. 10.23 Trapping and activation of antigen-specific naive T cells in lymphoid tissue. Naive T cells enter the lymph node from the blood and encounter many antigen-presenting dendritic cells in the lymph node cortex. T cells that do not recognize their specific antigen in the cortex leave via the efferent lymphatics and re-enter the blood. T cells that do recognize their specific antigen bind stably to the dendritic cell and are activated through their T-cell receptors, resulting in the production of armed effector T cells. Lymphocyte recirculation and recognition is so effective that all the specific naive T cells can be trapped by antigen in one node within 2 days. By 5 days after the arrival of antigen, activated effector T cells are leaving the lymph node in large numbers via the efferent lymphatics.

cells, they eventually leave the node via an efferent lymphatic vessel, which returns them to the blood so that they can recirculate through other lymph nodes. Rarely, a naive T cell recognizes its specific peptide:MHC complex on the surface of a dendritic cell, which signals the activation of LFA-1, causing the T cell to adhere strongly to the dendritic cell and cease migrating. Binding to the peptide:MHC complex and to co-stimulatory molecules on the surface of the dendritic cell also activates the naive T cell to proliferate and differentiate, resulting in the production of armed, antigen-specific effector T cells (see Fig. 8.2). The efficiency with which T cells screen each antigen-presenting cell in lymph nodes is very high, as can be seen by the rapid trapping of antigen-specific T cells in a single lymph node containing antigen: all antigen-specific T cells can be trapped in a lymph node within 48 hours of antigen deposition (Fig. 10.23).

10-17 Cytokines made in the early phases of an infection influence the functional differentiation of CD4 T cells.

It is during the initial response of naive CD4 T cells to antigen in the peripheral lymphoid tissues that the differentiation of these cells into the two major classes of CD4 effector T cell occurs. This step, at which a naive CD4 T cell becomes either an armed T_H1 cell or an armed T_H2 cell, has a critical impact on the outcome of an adaptive immune response, determining whether it will be dominated by macrophage activation or by antibody production. This is one of the most important events in the induction of an adaptive immune response.

The mechanism controlling this step in CD4 T-cell differentiation is not yet fully defined; however, it is clear that it can be profoundly influenced by cytokines present during the initial proliferative phase of T-cell activation. Experiments *in vitro* have shown that CD4 T cells initially stimulated in the presence of IL-12 and IFN-γ tend to develop into T_H1 cells (Fig. 10.24, left panels), in part because IFN-γ inhibits the proliferation of T_H2 cells. As IL-12 and IFN-γ are produced by dendritic cells, macrophages, and NK cells in the early phases of responses to viruses and some intracellular bacteria such as *Listeria* species, T-cell responses in these infections tend to be dominated by T_H1 cells. By contrast, CD4 T cells activated in the presence of IL-4 and especially IL-6 tend to differentiate into T_H2 cells, as IL-4 and IL-6 promote the differentiation of T_H2 cells, whereas IL-4 and IL-10 inhibit the generation of T_H1 cells.

One possible source of the IL-4 needed to generate T_H2 cells is a specialized subset of CD4 T cells that express the NK1.1 marker normally associated with NK cells and are called **NK1.1 CD4 T cells**. These T cells have a nearly invariant α:β T-cell receptor; in fact, essentially the same receptor seems to be used in the NK 1.1 CD4 T cells of mice and their counterparts in humans. Unlike other CD4 T cells, the development of the NK 1.1 CD4 T cells does not depend on the expression of MHC class II molecules. Instead, they recognize an MHC class IB molecule, CD1, which is not encoded within the MHC. In mice there are two CD1 genes (CD1.1 and CD1.2), whereas in humans there are five (CD1a–e) of which only CD1d is homologous to the murine CD1.1 and CD1.2. CD1 molecules are expressed by thymocytes, antigen-presenting cells, and intestinal epithelium.

Although the exact function of CD1 molecules is not well defined, CD1b is known to present a bacterial lipid, mycolic acid, to α:β T cells, whereas other CD1 molecules are recognized by γ:δ T cells. The activation of NK1.1 CD4 T cells is thought to depend on the expression of CD1 molecules induced in

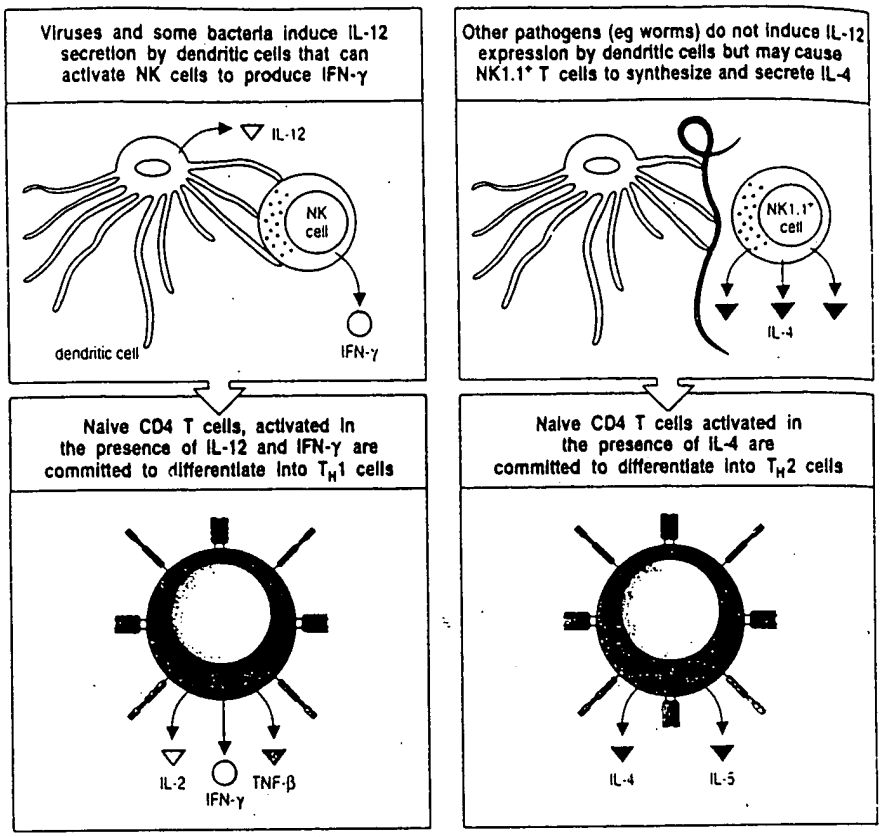


Fig. 10.24 The differentiation of naive CD4 T cells into armed effector cell types is influenced by cytokines elicited by the pathogen. Many pathogens, especially intracellular bacteria and viruses, activate dendritic cells and NK cells to produce IL-12 and IFN-γ, which act on proliferating CD4 T cells, causing them to differentiate into T_H1 cells. IL-4 can inhibit these responses. IL-4, produced by an NK1.1$^+$ CD4 T cell in response to parasitic worms or other pathogens, acts on proliferating CD4 T cells to cause them to become T_H2 cells. The mechanism by which these cytokines induce the selective differentiation of CD4 T cells is not known. They could act either when the CD4 T cell is first activated by an antigen-presenting cell or during the proliferative phase that ensues.

response to infection; whether the NK1.1 CD4 T cells recognize a specific antigen presented by these CD1 molecules is not known. Upon activation, these NK1.1 CD4 T cells secrete very large amounts of IL-4 and can therefore enhance the development of T_H2 cells (see Fig. 10.24, right panels), which promotes the production of IgG1 (mouse) and IgE (mouse, human) in subsequent humoral immune responses.

The differential capacity of pathogens to interact with dendritic cells, macrophages, NK cells, and NK1.1 CD4 T cells can therefore influence the overall balance of cytokines present early in the immune response and thus determine whether T_H1 or T_H2 cells develop preferentially to bias the adaptive immune response towards a cellular or a humoral response. This can in turn determine whether the pathogen is eliminated or survives within the host, and some pathogens might have evolved to interact with the innate immune system so as to generate responses that are beneficial to them rather than to the host.

10-18 Distinct subsets of T cells can regulate the growth and effector functions of other T-cell subsets.

The two subsets of CD4 T cells—T_H1 cells and T_H2 cells—have very different functions: T_H2 cells are the most effective activators of B cells, especially in primary responses, whereas T_H1 cells are crucial for activating macrophages. It is also clear that the two CD4 T-cell subsets can regulate each other: once one subset becomes dominant, it is often hard to shift the response to the other subset. One reason for this is that cytokines from one type of CD4 T cell

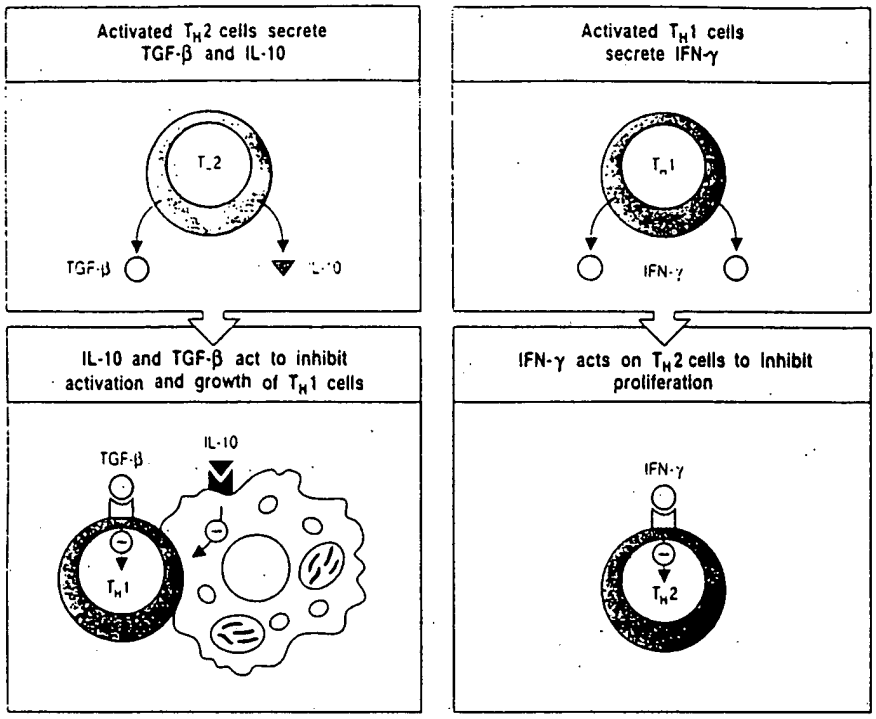


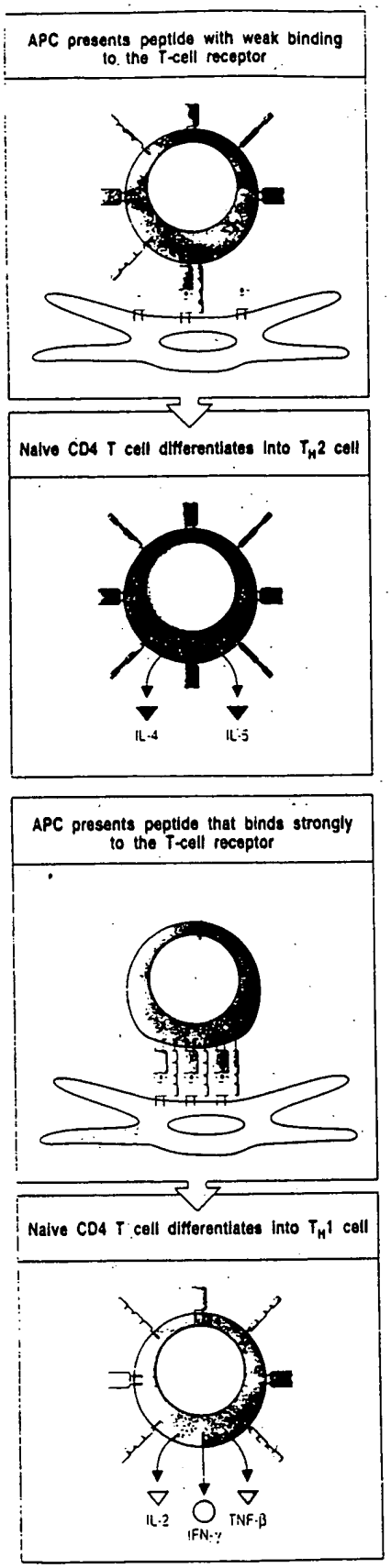
Fig. 10.25 The two subsets of CD4 T cells each produce cytokines that can negatively regulate the other subset. T_H2 cells make IL-10, which acts on macrophages to inhibit T_H1 activation, perhaps by blocking macrophage IL-12 synthesis, and TGF-β, which acts directly on the T_H1 cells to inhibit their growth (left panels). T_H1 cells make IFN-γ, which blocks the growth of T_H2 cells (right panels). These effects allow either subset to dominate a response by suppressing outgrowth of cells of the other subset.

inhibit the activation of the other. Thus, IL-10, a product of T_H2 cells, can inhibit the development of T_H1 cells by acting on the antigen-presenting cell, whereas IFN-γ, a product of T_H1 cells, can prevent the activation of T_H2 cells (Fig. 10.25). If a particular CD4 T-cell subset is activated first or preferentially in a response, it can suppress the development of the other subset. The overall effect is that certain responses are dominated by either humoral (T_H2) or cell-mediated (T_H1) immunity.

This interplay of cytokines is important in human disease, but it has been explored at present mainly in certain mouse models, where such polarized responses are easier to study. For example, when CD4 T cells in BALB/c mice are stimulated with the protozoan parasite *Leishmania*, their CD4 T cells fail to differentiate into T_H1 effector cells; instead, they preferentially make T_H2 cells in response to this pathogen. These T_H2 cells are unable to activate macrophages to inhibit leishmanial growth, resulting in susceptibility to disease. By contrast, C57BL/6 mice respond by producing T_H1 cells that protect the host by activating infected macrophages to kill the *Leishmania*. The activation of T_H2 cells in BALB/c mice can be reversed if IL-4 is blocked in the first days of infection by injecting anti-IL-4 antibody. This treatment is ineffective after a week or so of infection.

Because cytokines seem to regulate the balance between T_H1 and T_H2 cells, one might expect that it would be possible to shift this balance by administering appropriate cytokines. IL-2 and IFN-γ have been used to stimulate cell-mediated immunity in diseases such as lepromatous leprosy and can cause both a local resolution of the lesion and a systemic change in T-cell responses, as we shall see in Chapter 11. IL-12, which is a potent inducer of T_H1 cells, might be an even more attractive potential therapy.

CD8 T cells are also able to regulate the immune response by producing cytokines. It has become clear recently that CD8 T cells can, in addition to their familiar cytolytic function, also respond to antigen by secreting



cytokines typical of either T_H1 or T_H2 cells. Such CD8 T cells, called T_C1 or T_C2 by analogy to the T_H subsets, seem to be responsible for the development of leprosy in its lepromatous rather than its tuberculoid form, which we discuss in detail in Chapter 11 (see Fig. 11.6). Patients with the less destructive tuberculoid leprosy make only T_H1 cells, which can activate macrophages to rid the body of leprosy bacilli. Patients with lepromatous leprosy have CD8 T cells that suppress the T_H1 response by making IL-10 and the cytokine tumor growth factor (TGF)-β. Thus, the suppression by CD8 T cells that has been observed in various situations can be explained by their expression of different sets of cytokines.

10-19 The nature and amount of antigenic peptide can also affect the differentiation of CD4 T cells.

Another factor that influences the differentiation of CD4 T cells into distinct effector subsets is the amount and exact sequence of the antigenic peptide that initiates the response. Large amounts of peptides that achieve a high density on the surface of antigen-presenting cells tend to stimulate T_H1 cell responses, whereas low-density presentation tends to elicit T_H2 cell responses. Moreover, peptides that interact strongly with the T-cell receptor tend to stimulate T_H1-like responses, whereas peptides that bind weakly tend to stimulate T_H2-like responses (Fig. 10.26).

This difference could be very important in several circumstances. For instance, allergy is caused by the production of IgE antibody, which, as we learned in Chapter 9, requires high levels of IL-4 but does not occur in the presence of IFN-γ, a potent inhibitor of IL-4-driven class switching to IgE. We shall see in Chapter 12 that antigens that elicit IgE-mediated allergy are generally delivered in minute doses, and that they elicit T_H2 cells that make IL-4 and no IFN-γ. It is also relevant that allergens do not elicit any of the known innate immune responses, which produce cytokines that tend to bias CD4 T-cell differentiation toward T_H1 cells.

Most protein antigens that elicit CD4 T-cell responses stimulate the production of both T_H1 and T_H2 cells. This reflects the presence in most proteins of several different peptide sequences that can bind to MHC class II molecules and be presented to T cells. Some of these peptides are likely to bind to MHC class II molecules with high affinity, and consequently might be present at high density on the antigen-presenting cell, whereas others might bind with low affinity and be present only at low density. Naive T cells specific for peptide antigens that have high affinity for MHC molecules are therefore likely to encounter a high density of their ligand, whereas others might only encounter a low density, and these differences in ligand density might affect the subsequent response of the T cell. Indeed, it can be demonstrated experimentally that some peptides in a protein tend to elicit T_H2 cells, whereas others tend to elicit T_H1 cells.

Fig. 10.26 The nature and amount of ligand presented t a CD4 T cell during primary stimulation can determine its functi nal phenotype. CD4 T cells presented with low levels of a ligand that binds the T-cell receptor poorly differentiate preferentially into T_H2 cells making IL-4 and IL-5. Such T cells are most active in stimulating naive B cells to differentiate into plasma cells and make antibody. T cells presented with a high density of a ligand that binds the T-cell receptor strongly differentiate into T_H1 cells that secrete IL-2, TNF-β, and IFN-γ, and are most effective in activating macrophages.

10-20 Armed effector T cells are guided to sites of infection by newly expressed surface molecules.

The full activation of naive T cells takes 4–5 days and is accompanied by marked changes in the homing behavior of these cells. These occur because of changes in the expression of several cell-surface adhesion molecules that direct the migration of T cells. Thus, many armed effector T cells lose expression of the L-selectin molecule that mediates homing to the lymph nodes, whereas the expression of other adhesion molecules is increased (Fig. 10.27). One important change is a marked increase in the expression of the α_4 integrin VLA-4 (see Section 8-2), which binds to vascular cell adhesion molecule-1 (VCAM-1); cytokines induce the expression of VCAM-1 on endothelial cells at sites of infection in peripheral tissues. In this way, newly differentiated armed effector T cells are directed to the site of infection in the peripheral tissues.

Differential expression of adhesion molecules can also direct different subsets of armed effector T cells to specific sites. Some, for example, migrate to the lamina propria of the gut, which involves the binding of both L-selectin and the $\alpha_4\beta_7$ integrin expressed on the T cell to separate sites on MAdCAM-1. T cells that home to the epithelium of the gut express a novel integrin called $\alpha_e\beta_7$ and bind to E-cadherin expressed on epithelial cells. Cells that home to the skin, by contrast, express the cutaneous lymphocyte antigen (CLA) and bind to E-selectin.

Not all infections trigger innate immune responses that activate local endothelial cells, and it is not so clear how T cells are guided to the sites of infection in these cases. Armed effector T cells seem to enter all tissues in very small numbers, perhaps via adhesive interactions such as the binding of LFA-1 to ICAM-2, which is constitutively expressed on all endothelial cells. If these T cells recognize specific antigen in the tissue they enter, they produce cytokines such as TNF-α, which activates endothelial cells to express E-selectin, VCAM-1, and ICAM-1, and chemokines such as RANTES (see Fig. 10.13), which can then act on effector T cells to activate their adhesion molecules. The increased levels of VCAM-1 and ICAM-1 on endothelial cells bind VLA-4 and LFA-1, respectively, on armed effector T cells, recruiting more of these cells into tissues that contain antigen. At the same time, monocytes and polymorphonuclear leukocytes are recruited to these sites by adhesion to E-selectin. The TNF-α and IFN-γ released by the activated T cells also act synergistically to change the shape of endothelial cells, allowing increased blood flow, increased vascular permeability, and increased emigration of leukocytes, fluid, and protein into a site of infection.

Thus, one or a few specific effector T cells encountering antigen in a tissue can initiate a potent local inflammatory response that recruits both more specific effector cells and many accessory cells to that site. Most of the armed effector T cells that migrate at random into tissues will of course not

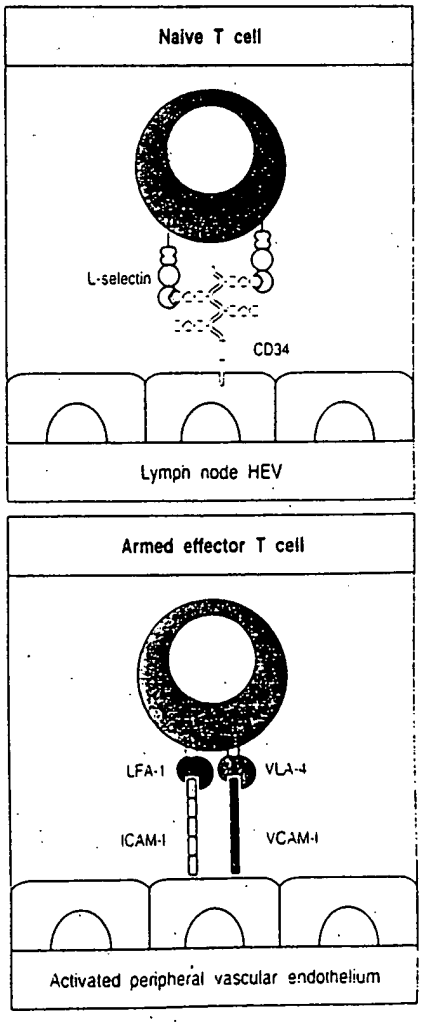


Fig. 10.27 Armed effector T cells change their surface molecules so that they can home to sites of infection via the blood. Naive T cells home to lymph nodes through the binding of L-selectin to sulfated carbohydrates displayed by various proteins, such as CD34 and GlyCAM-1 (upper panel). If they encounter antigen and differentiate into effector cells, many lose expression of L-selectin, leave the lymph node about 4–5 days later, and now express VLA-4 and increased levels of LFA-1. These bind to VCAM-1 and ICAM-1 respectively on peripheral vascular endothelium at sites of inflammation (lower panel). HEV, high endothelial venule.

encounter specific antigen, and these cells either enter afferent lymph and return to the bloodstream, or undergo apoptotic death in the tissues. Most of the T cells in afferent lymph draining peripheral tissues are memory or effector T cells that express CD45R0 and lack L-selectin. They seem to be committed to migration through potential sites of infection.

10-21 Antibody responses develop in lymphoid tissues under the direction of armed helper T cells.

Migration into the periphery is clearly important for the effector actions of CD8 cytotoxic T cells, and for T_H1 cells, which need to activate macrophages at the site of an infection. However, the most important functions of helper T cells, and of T_H2 cells in particular, depend on their interactions with B cells, and these interactions occur in the lymphoid tissues themselves. B cells specific for protein antigens cannot be activated to proliferate, form germinal centers, or differentiate into plasma cells until they encounter a helper T cell that is specific for one of the peptides derived from that antigen or antigenic complex. It follows, therefore, that humoral immune responses to protein antigens cannot occur until after antigen-specific helper T cells have been generated.

One of the most interesting questions in immunology is how two antigen-specific lymphocytes, the naive antigen-binding B cell and the armed helper T cell, find one another to initiate a T-cell dependent antibody response. As we learned in Chapter 9, the likely answer lies in the migratory path of B cells through the lymphoid tissues and the presence of armed helper T cells on that path (Fig. 10.28).

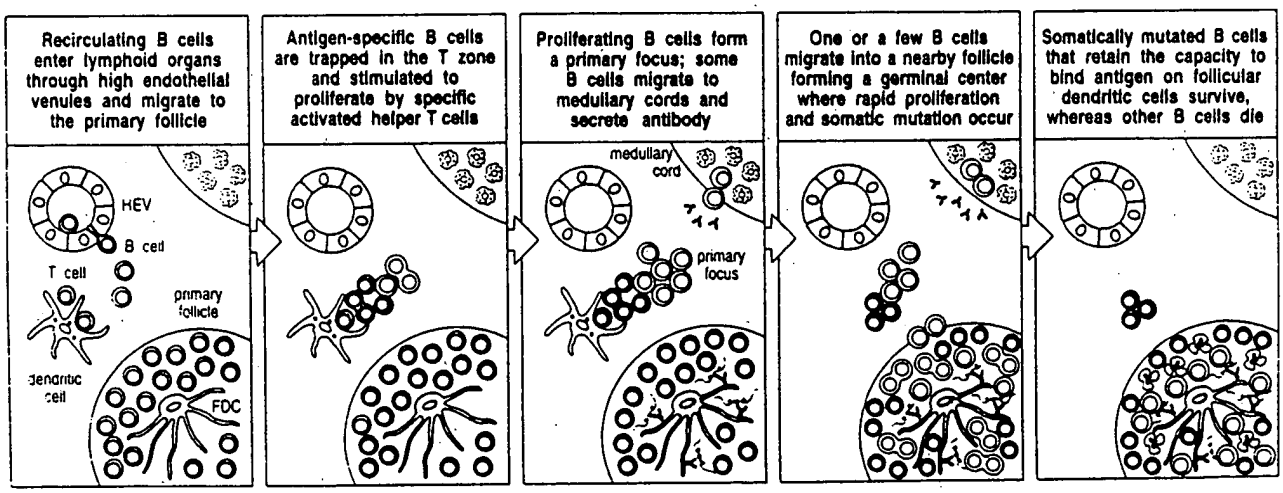


Fig. 10.28 The specialized regions of lymphoid tissue provide an environment where antigen-specific B cells can interact with armed helper T cells specific for the same antigen. The initial encounter of antigen-specific B cells with the appropriate helper T cells occurs in the T-cell areas in lymphoid tissue and stimulates the proliferation of B cells in contact with the helper T cells, resulting in some isotype switching. Some activated B-cell blasts then migrate to medullary cords, where they divide, differentiate into plasma cells, and secrete antibody for a few days. Other cells migrate into primary lymphoid follicles where they proliferate rapidly to form a germinal center under the influence of antigen trapped by follicular dendritic cells (FDC) and of helper T cells. The germinal center is the site of somatic hypermutation and selection of high-affinity B cells on the FDC network. In the primary response, antigen trapping is thought to occur only after initial production of antibody by B cells in the primary focus and the medullary cords. HEV, high endothelial venule.

B cells migrate through peripheral lymphoid organs in much the same way as T cells (see Fig. 10.28, first panel), and it is thought that the trapping and activation of naive CD4 T cells in the T-cell areas of lymphoid tissues provides a concentration of antigen-specific helper T cells capable of activating those rare B cells that are specific for the same antigen. Antigen-specific B cells are also enriched in these same areas by binding their cognate antigen; such cells are observed to accumulate in T-cell areas of the spleen and lymph nodes when exposed to their specific antigen. If the B cells receive specific signals from armed helper T cells, they proliferate in the T-cell areas of lymphoid tissues (see Fig. 10.28, second panel). In the absence of T-cell signals, these antigen-specific B cells die in less than 24 hours after arriving in the T cell zone.

About 5 days after primary immunization, primary foci of proliferating B cells appear in the T-cell areas, which correlates with the time needed for helper T cells to differentiate. Some of the B cells activated in the primary focus may migrate to the medullary cords of the lymph node or to those parts of the red pulp that are next to the T-cell zones of the spleen and secrete specific antibody for a few days (see Fig. 10.28, third panel). Others migrate to the follicle (see Fig. 10.28, fourth panel), where they proliferate further, forming a germinal center in which they undergo somatic hypermutation (see Chapter 9). The antibodies secreted by B cells differentiating early in the response not only provide early protection; they may also be important in trapping antigen, in the form of antigen:antibody complexes, on the surface of the local follicular dendritic cells. This contributes to the selection of B cells by antigen that underlies the affinity maturation observed during an antibody response. The antigen is held by a non-phagocytic Fc receptor on the follicular dendritic cells in the form of antigen:antibody complexes. Antigen can be retained in lymphoid follicles in this form for very long periods.

The proliferation, somatic hypermutation, and selection that occur in the germinal centers during a primary antibody response have been described in Chapter 9. The adhesion molecules that govern the migratory behavior of B cells are likely to be very important to this process but, as yet, little is known of their nature or of the ligands to which they bind.

10-22 Antibody responses are sustained in medullary cords and bone marrow.

Antibody-secreting cells are generated either as the result of B cells' proliferating in primary foci or after the migration of B cells to follicles. The proliferative response is started by B cells that have taken up antigen interacting in the T-cell zone with helper T cells specific for the same antigen. The B cells activated in primary foci then migrate either to adjacent follicles or to local extrafollicular sites of proliferation. The extrafollicular sites in lymph nodes are the medullary cords and in the spleen are those parts of the red pulp directly adjoining the T-cell zone. B cells grow exponentially in these sites for 2–3 days and undergo six or seven cell divisions before the progeny come out of the cell cycle and form antibody-producing plasma cells *in situ*. Most of these plasma cells have a life-span of 2–3 days, after which they undergo apoptosis. About 10% of plasma cells in these extrafollicular sites live longer; their origin and ultimate fate are unknown (see Fig. 10.29, lower panel). B cells that migrate to the primary follicles to form germinal centers have been discussed in Chapter 9 (see Sections 9-6 to 9-8). Some B cells leave germinal centers as plasmablasts (pre-plasma cells). Plasmablasts originating in the follicles of Peyer's patches and mesenteric lymph nodes migrate via lymph and

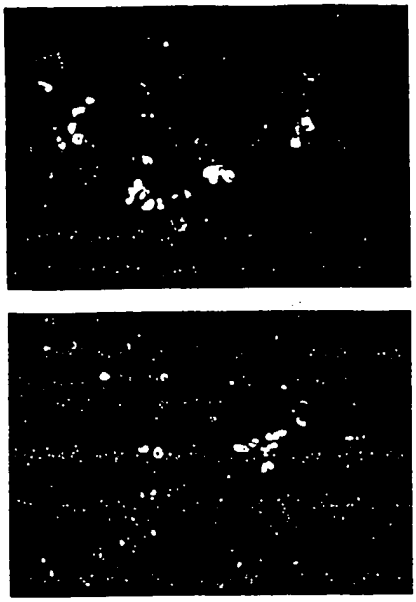


Fig. 10.29 Plasma cells are dispersed in medullary cords and b ne marr w. In these sites they secrete antibody at high rates directly into the blood for distribution to the rest of the body. In the upper micrograph, longer-lived plasma cells (3 weeks to 3 months or more) in the bone marrow are revealed with antibodies specific for light chains (fluorescein anti-λ and rhodamine anti-κ stain). Plasma cells secreting immunoglobulins containing λ light chains stain green, whereas those secreting immunoglobulins containing κ light chains stain red. In the lower micrograph, plasma cells in lymph node medullary cords are stained green (with fluorescein anti-IgA) if they are secreting IgA, and red (with rhodamine anti-IgG) if they are secreting IgG. These plasma cells are short lived (2–4 days). The lymphatic sinuses are outlined by granular staining selective for IgA. Photographs courtesy of P Bramdtzaeg.

blood to the lamina propria of the gut and other epithelial surfaces. Those originating in peripheral lymph node or splenic follicles migrate to the bone marrow (see Fig. 10.29, upper panel). In these distant sites of antibody production, the plasmablasts differentiate into plasma cells that mostly have a life span of about 1 month, though a fraction of these cells can persist for much longer.

Studies of the responses to non-replicating antigens show that germinal centers are present for only 3–4 weeks after the supply of extrafollicular antigen has been exhausted. Small numbers of B cells, however, continue to proliferate in the follicles for months, and are likely to be the precursors of antigen-specific plasma cells in the mucosa and bone marrow throughout the subsequent months and years.

10-23 The effector mechanisms used to clear an infection depend on the infectious agent.

A primary adaptive immune response to an infection serves to clear the primary infection from the body and to provide protection against re-infection with the same pathogen in most cases. However, some pathogens evade complete clearance and persist for the life of the host, for example, *Leishmania*, toxoplasma, and herpes viruses. Fig. 10.30 summarizes the different types of infection and the ways in which they can be eliminated effectively by an initial adaptive immune response.

Immunity to re-infection is called **protective immunity**, and inducing protective immunity is the goal of vaccine development. Protective immunity consists of two components, immune reactants generated in the initial infection or by vaccination, and long-lived immunological memory (Fig. 10.31), which we shall consider in the last part of this chapter. Protective immunity might require the presence of preformed reactants, such as antibody molecules or armed effector T cells. For instance, effective protection against polio virus requires pre-existing antibody, because the virus will rapidly infect motor neurons and lead to their destruction unless it is neutralized by antibody and prevented from spreading within the body. Specific IgA on epithelial surfaces can also neutralize the virus before it enters the body. Thus, protective immunity can involve effector mechanisms (IgA in this case) that do not operate in the elimination of the primary infection. Pre-formed reactants can also allow the immune system to respond more rapidly and efficiently to a second exposure to a pathogen. Thus, when antibody is present, opsonization and phagocytosis of pathogens will be more efficient. If specific IgE is present, then pathogens will also be able to activate mast cells, rapidly initiating an inflammatory response through the release of histamine and leukotrienes.

	Infectious agent	Disease	Humoral immunity				Cell-mediated immunity	
			IgM	IgG	IgE	IgA	CD4 T cells (macrophages)	CD8 killer T cells
Viruses	Variola	Smallpox						
	Varicella zoster	Chickenpox						
	Epstein–Barr virus	Mononucleosis						
	Influenza virus	Influenza						
	Mumps virus	Mumps						
	Measles virus	Measles						
	Polio virus	Poliomyelitis						
	Human immunodeficiency virus	AIDS						
Bacteria	*Staphylococcus aureus*	Boils						
	Streptococcus pyogenes	Tonsilitis						
	Streptococcus pneumoniae	Pneumonia						
	Neisseria gonorrhoeae	Gonorrhea						
	Neisseria meningitidis	Meningitis						
	Corynebacterium diphtheriae	Diphtheria						
	Clostridium tetani	Tetanus						
	Treponema pallidum	Syphilis			Transient			
	Borrelia burgdorferi	Lyme disease			Transient			
	Salmonella typhi	Typhoid						
	Vibrio cholerae	Cholera						
	Legionella pneumophila	Legionnaire's disease						
	Rickettsia prowazeki	Typhus						
	Chlamydia trachomatis	Trachoma						
	Mycobacteria	Tuberculosis, leprosy						
Fungi	*Candida albicans*	Candidiasis						
Protozoa	*Plasmodium* spp.	Malaria						
	Toxoplasma gondii	Toxoplasmosis						
	Trypanosoma spp.	Trypanosomiasis						
	Leishmania spp.	Leishmaniasis						
Worms	Schistosome	Schistosomiasis						

Fig. 10.30 Different effector mechanisms are used to clear primary infections with different pathogens and to protect against subsequent re-infection. The pathogens are listed in order of increasing complexity, and the defense mechanisms used to clear a primary infection are identified by the red shading of the boxes where these are known. Yellow shading indicates a role in protective immunity. Paler shades indicate less well-established mechanisms. Much has to be learned about such host–pathogen interactions. It is clear that classes of pathogens elicit similar protective immune responses, reflecting similarities in their lifestyles.

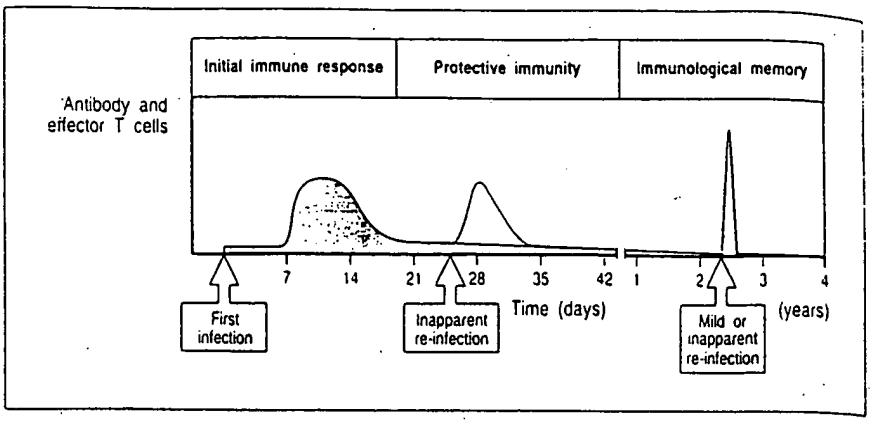


Fig. 10.31 Protective immunity consists of preformed immune reactants and immunological memory. Antibody levels and effector T-cell activity gradually decline after an infection is cleared. An early re-infection is rapidly cleared by these immune reactants. There are few symptoms but levels of immune reactants increase. Re-infection at later times leads to rapid increases in antibody and effector T cells owing to immunological memory, and infection can be mild or even inapparent.

Summary.

The adaptive immune response is required for effective protection of the host against pathogenic microorganisms. Adaptive immune responses occur when pathogens have overwhelmed or evaded non-adaptive mechanisms of host defense and established a focus of infection. The antigens of the pathogen are transported to local lymphoid organs by migrating antigen-presenting cells. This antigen is processed and presented to antigen-specific naive T cells that continuously recirculate through the lymphoid organs. T-cell priming and the differentiation of armed effector T cells occurs here, and the armed effector T cells either leave the lymphoid organ to effect cell-mediated immunity in sites of infection in the tissues, or remain in the lymphoid organ to participate in humoral immunity by activating antigen-binding B cells. Which response occurs is determined by the differentiation of CD4 T cells into T_H1 or T_H2 cells, which is in turn determined by the cytokines produced in the early non-adaptive phase. CD4 T-cell differentiation is also affected by ill-defined characteristics of the activating antigen and by its overall abundance. Ideally, the adaptive immune response eliminates the infectious agent and provides the host with a state of protective immunity against re-infection with the same pathogen.

Immunological memory.

Perhaps the most important consequence of an adaptive immune response is the establishment of a state of immunological memory. Immunological memory is the ability of the immune system to respond more rapidly and effectively to pathogens that have been encountered previously, and reflects the pre-existence of a clonally expanded population of antigen-specific lymphocytes. Memory responses, which are called secondary, tertiary, and so on, depending on the number of exposures to antigen, also differ qualitatively from primary responses.

This is particularly clear in the case of the antibody response, where the characteristics of antibodies produced in secondary and subsequent responses are distinct from those produced in the primary response to the same antigen. How immunological memory is maintained, however, is still

poorly understood. The principal focus of this section will therefore be the altered character of memory responses, although we shall also outline the mechanisms that have been suggested to explain the persistence of immunological memory after exposure to antigen.

10-24 Immunological memory is long-lived after infection or vaccination.

Most children in the United States are now vaccinated against measles virus: before vaccination was widespread, most were naturally exposed to this virus and suffered from an acute, unpleasant, and potentially dangerous viral illness. Whether through vaccination or through infection, children exposed to the virus acquire long-term protection from measles. The same is true of many other acute infectious diseases: this state of protection is a consequence of immunological memory.

The basis of immunological memory has been hard to explore experimentally; although the phenomenon was first recorded by the ancient Greeks and has been exploited routinely in vaccination programs for over 200 years, it is still not clearly established whether memory reflects a long-lived population of specialized memory cells or depends on the persistence of undetectable levels of antigen that continuously re-stimulate antigen-specific lymphocytes. It can be demonstrated, however, that only individuals who were themselves previously exposed to a given infectious agent are immune, and that memory is not dependent on repeated exposure to infection as a result of contacts with other infected individuals. This was established by observations on remote island populations, where a virus such as measles can cause an epidemic, infecting all people living on the island at that time, after which the virus disappears for many years. On reintroduction from outside the island, the virus does not affect the original population but causes disease in those people born since the initial epidemic. This means that immunological memory cannot be caused by repeated exposure to infectious virus and leaves two alternative explanations.

The first is that memory is sustained by long-lived lymphocytes, induced by the original exposure, that persist in a resting state until a second encounter with the pathogen. The second is that the lymphocytes activated by the original exposure to antigen are repeatedly restimulated, even in the absence of re-infection with the pathogen. This could occur in several ways. One possibility is that the pathogen persists in small amounts that are sufficient to restimulate the activated cells but not to spread the infection to others; for example restimulation might occur through the persistence of pathogen antigens in immune complexes bound to follicular dendritic cells. Another possibility is that restimulation occurs through exposure to other, cross-reactive, antigens; these might be able to stimulate previously activated lymphocytes specific for the pathogen even though they would not have activated their naive precursor cells. Finally, restimulation could also be mediated by cytokines produced during the course of antigen-specific immune responses directed at non cross-reactive antigens; bystander memory cells, but not naive cells, might be stimulated in this way.

The experimental measurement of immunological memory has been carried out in various ways. Adoptive transfer assays of lymphocytes from animals immunized with simple, non-living antigens have been favored for such studies, as the antigen cannot proliferate. When an animal is immunized with a protein antigen, helper T cell memory appears abruptly and at its maximal level after 5 days or so. Antigen-specific memory B cells appear some days

later, because B-cell activation cannot begin until armed helper T cells are available, and B cells must then enter a phase of proliferation and selection in lymphoid tissue. By 1 month after immunization, memory B cells will be present at their maximal levels. These levels are then maintained with little alteration for the lifetime of the animal. In these experiments, the existence of memory cells is measured purely in terms of the transfer of specific responsiveness from an immunized or 'primed' animal to an irradiated, immuno-incompetent host (see Sections 2-20 and 2-23). In succeeding sections, we shall look in more detail at the changes that occur in lymphocytes after antigen priming and discuss the mechanisms that might account for these changes.

10-25 Both clonal expansion and clonal differentiation contribute to immunological memory in B cells.

Immunological memory in B cells can be examined by isolating B cells from immunized mice and restimulating them with antigen in the presence of armed helper T cells specific for the same antigen. In this way, it is possible to show that antigen-specific memory B cells differ both quantitatively and qualitatively from naive B cells. B cells that can respond to antigen increase in frequency after priming by about 10- to 100-fold (Fig. 10.32) and produce antibody of higher average affinity than unprimed B lymphocytes; the affinity of that antibody continues to increase during the ongoing secondary and subsequent antibody responses (Fig. 10.33). The secondary antibody response is characterized in its first few days by the production of small amounts of IgM antibody and larger amounts of IgG antibody, with some IgA and IgE. These antibodies are produced by memory B cells that have already switched from IgM to these more mature isotypes and express IgG, IgA, or IgE on their surface, as well as a somewhat higher level of MHC class II molecules than is characteristic of naive B cells. Increased affinity for antigen and increased levels of MHC class II expression facilitate antigen uptake and presentation, and allow memory B cells to initiate their critical interactions with armed helper T cells at lower doses of antigen. Recent evidence from mice injected with antibody against nerve growth factor shows a profound loss of the capacity to make IgG antibody in a secondary response. These results suggest a role for this hormone in sustaining B-cell memory.

The distinction between primary and secondary antibody responses is most clearly seen in those cases where the primary response is dominated by antibodies that are closely related and show few if any somatic hypermutations.

	Source of B cells	
	Unimmunized donor Primary response	Immunized donor Secondary response
Frequency of specific B cells	$1:10^4 - 1:10^5$	$1:10^3$
Isotype of antibody produced	IgM > IgG	IgG, IgA
Affinity of antibody	Low	High
Somatic hypermutation	Low	High

Fig. 10.32 The generation of secondary antibody responses from memory B cells is distinct from the generation of the primary antibody response. The primary response usually consists of antibody molecules made by plasma cells derived from a relatively large number of different precursor B cells. The antibodies are of relatively low affinity with few somatic mutations. The secondary response derives from far fewer, high-affinity precursor B cells, which have undergone significant clonal expansion. Their receptors and antibodies are of high affinity for the antigen and show extensive somatic mutation. Thus, there is usually only a 10- to 100-fold increase in the frequency of activatable B cells after priming; however, the quality of the antibody response is altered radically, such that these precursors induce a far more intense and effective response.

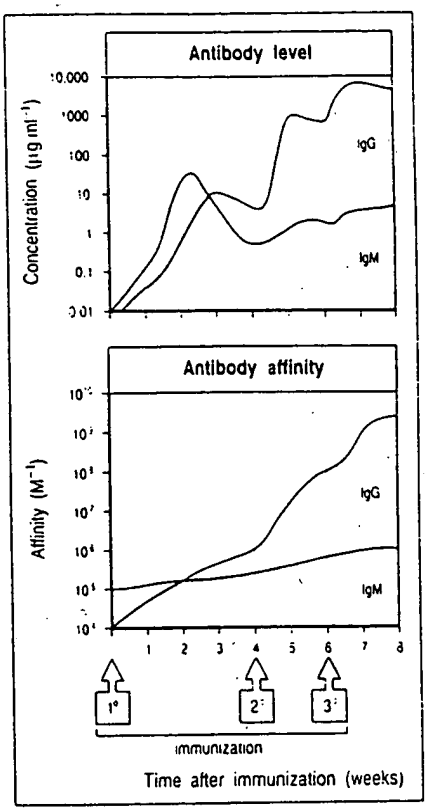


Fig. 10.33 The affinity as well as the amount of antibody increase with repeated immunization. The upper panel shows the increase in the level of antibody with time after primary, followed by secondary and tertiary, immunization; the lower panel shows the increase in the affinity of the antibodies. The increase in affinity (affinity maturation) is seen largely in IgG antibody (as well as in IgA and IgE, which are not shown) coming from mature B cells that have undergone isotype switching and somatic hypermutation to yield higher-affinity antibodies. Although some affinity maturation occurs in the primary antibody response, most arises in later responses to repeated antigen injections. Note that these graphs are on a logarithmic scale.

This occurs in inbred mouse strains in response to certain haptens that might by chance activate a pre-existing set of naive B cells poised to respond to such antigens. Such antibodies are encoded by the same V_H and V_L genes in all animals of the strain, suggesting that these variable regions might have been selected during evolution for recognition of determinants on pathogens that happen to cross-react with some haptens. As a result of the uniformity of these primary responses, changes in the antibody molecules produced in secondary responses to the same antigens are easy to observe. These differences include not only numerous somatic mutations in antibodies containing the dominant variable regions but also the addition of antibodies containing V_H and V_L gene segments not detected in the primary response. These are thought to derive from B cells that were activated at low frequency during the primary response (and thus were not detected) and differentiated into memory B cells.

10-26 Repeated immunizations lead to increasing affinity of antibody owing to somatic hypermutation and selection by antigen in germinal centers.

As we saw in Section 10-21 and Fig. 10.28, in a primary antibody response naive B cells stimulated by armed helper T cells form a primary extrafollicular focus in lymphoid tissues, where some differentiate and secrete antibody that helps to localize antigen on the surface of follicular dendritic cells (Fig. 10.34). Some B cells that have not yet undergone terminal differentiation migrate into the follicle and become germinal center B cells. Stimulated by the antigen-bearing follicular dendritic cells, these B cells enter a second proliferative phase, during which the DNA encoding their immunoglobulin variable domains undergoes somatic hypermutation before the B cells differentiate into antibody-secreting plasma cells (see Section 9-7).

The antibodies produced by plasma cells in the primary response have an important role in driving affinity maturation in the secondary response. In secondary and subsequent immune responses, any persisting antibodies produced by the B cells that differentiated in the primary response are immediately available to bind to the newly introduced antigen. Some of these antibodies divert antigen to phagocytes for degradation and disposal; however, some seem to be trapped by special antigen-transporting cells in the marginal zones of the spleen and the marginal sinus of lymph nodes. These cells bind antigen:antibody complexes and, instead of ingesting them, transport them to the lymphoid follicles, where the complexes are subsequently found on the surface of follicular dendritic cells. It is possible that the antigen-transporting cells in the spleen are B cells. In the lymph nodes, the transporter cells are resistant to ionizing radiation and their nature is obscure.

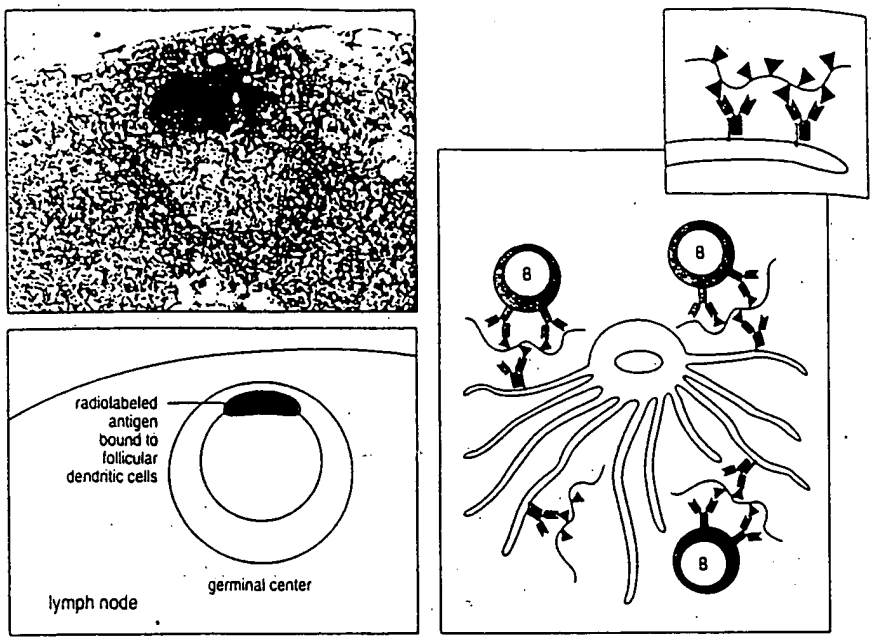


Fig. 10.34 B cells recognize antigen as immune complexes bound to the surface of follicular dendritic cells. Radiolabeled antigen localizes to, and persists in, lymphoid follicles of draining lymph nodes (see light micrograph and schematic representation below, showing a germinal center in a lymph node). Radiolabeled antigen has been injected 3 days previously and its localization in the germinal center is shown by the intense dark staining. The antigen is in the form of antigen:antibody:complement complexes bound to Fc and complement receptors on the surface of the follicular dendritic cell. These complexes are not internalized, as depicted schematically for antigen:antibody complexes bound to the Fc receptor in the right panel and insert. Antigen can persist in this form for long periods. Photograph courtesy of J Tew.

The follicular dendritic cells package the antigen into bundles of membrane coated with antigen:antibody complexes that bud off the follicular dendritic cell surface; these structures are called **iccosomes** (Fig. 10.35). It is believed that B cells whose receptors bind the antigen with sufficient avidity to compete with the existing antibody take up these iccosomes, process the antigen into peptide fragments, and present these peptides bound to MHC class II molecules to armed helper T cells surrounding and infiltrating the germinal centers (see Section 9-8). Contact between B cells presenting antigen fragments and armed helper T cells specific for the same peptides leads to an exchange of activating signals and the rapid proliferation of both

Fig. 10.35 Immune complexes bound to follicular dendritic cells form iccosomes, which are released and can be taken up by B cells in the germinal center. Follicular dendritic cells have a prominent cell body and many dendritic processes. Immune complexes, bound to Fc receptors on the follicular dendritic cell surface, become clustered, forming prominent 'beads' along the dendrites. An intermediate form of follicular dendritic cell is shown (left panel) with both straight filiform dendrites and those that are becoming beaded. These beads are shed from the cell as iccosomes (immune complex coated bodies), which can bind (center panel) and be taken up by B cells in the germinal center (right panel). In the center and right panels, the iccosome has been formed with immune complexes containing horseradish peroxidase, which is electron-dense and thus appears dark in the transmission electron micrographs. Photographs courtesy of A K Szakal.

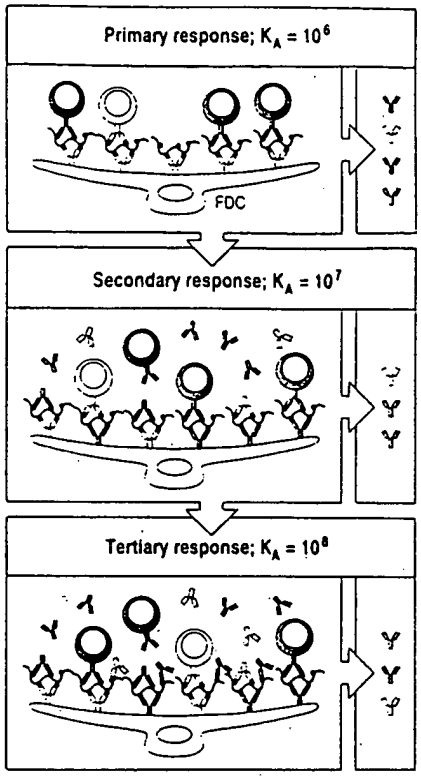


Fig. 10.36 The mechanism of affinity maturation in an antibody response. At the beginning of a primary response, high concentrations of antigen in the presence of small amounts of antibody lead to the formation of antigen:antibody complexes on follicular dendritic cells (FDCs), with little free antibody. B cells with receptors of a wide variety of affinities (K_A), most of which will bind antigen with low affinity, can thus interact with the FDCs, producing antibody of varying and relatively low affinity (top panel). Those B cells with receptors of the highest affinity are most efficient at extracting antigen from FDCs in germinal centers, and these are then selected to survive by interaction with helper T cells, even if the high-affinity B cells are actually quite infrequent. On the re-introduction of antigen, antibody produced in the primary response competes with B-cell receptors for binding to antigen:antibody complexes on FDCs, and in the secondary response only B cells with receptors of high enough affinity to compete with existing antibodies can bind antigen and contribute to the response (middle panel). In the tertiary response, the same mechanism selects for B-cell receptors with still higher affinity (bottom panel).

activated antigen-specific B cells and helper T cells. This process depends on bidirectional signaling through CD40L on the activated T cell and CD40 on the B cell, and on the induction of other co-stimulatory molecules. In this way, the affinity of the antibody produced rises progressively, as only B cells with high-affinity antigen receptors can bind antigen efficiently and be driven to proliferate by antigen-specific helper T cells (Fig. 10.36).

10-27 Memory T cells are increased in frequency and have distinct activation requirements and cell-surface proteins that distinguish them from armed effector T cells.

Because the T-cell receptor does not undergo isotype switching or affinity maturation, memory T cells have been more difficult to characterize than memory B cells. The number of T cells reactive to an antigen increases markedly after immunization, persisting at a level significantly (10- to 100-fold) above the initial frequency for the rest of the animal's or person's life. These cells carry cell-surface proteins more characteristic of armed effector cells than of naive T cells. However, it is not easy to establish whether these cells really are long-lived armed effector T cells, or whether they are cells with distinct properties that should be specifically designated memory T cells. This issue does not arise with B cells because effector B cells, as we saw in Chapter 9, are terminally differentiated plasma cells, many of which die between 3 days and 6 weeks after antigen exposure.

A major problem in experiments aimed at establishing the existence of memory T cells is that most assays for T-cell effector function take several days, during which the putative memory T cells are re-induced to armed effector cell status, so that the assays do not distinguish pre-existing effector cells from memory T cells. This problem does not apply to cytotoxic T cells, however, as cytotoxic effector T cells can program a target cell for lysis in 5 minutes. Memory CD8 T cells need to be re-activated to become cytotoxic, but they can be so without undergoing DNA synthesis, as shown by studies carried out in the presence of mitotic inhibitors. Recently it has become possible to enumerate CD8 T cells by staining them with tetrameric MHC:peptide complexes. It has been found that the number of antigen-specific CD8 T cells increases dramatically during an infection, and then drops by up to 100-fold; nevertheless, this level is distinctly higher than before priming. These experiments should soon elucidate the state of long-term memory cells.

The issue is more difficult to address for CD4 T-cell responses, and the identification of memory CD4 T cells rests largely on the existence of a population of cells with the surface characteristics of activated armed effector T cells (Fig. 10.37) but distinct from them in that they require additional restimulation before acting on target cells. Changes in three cell-surface proteins—L-selectin, CD44, and CD45—are particularly significant after exposure to antigen. L-selectin is lost on most memory T cells, whereas CD44 levels increase on all memory T cells after priming, and the isoform of CD45 changes because of alternative splicing of exons that encode the extracellular domain of CD45 (Fig. 10.38), leading to isoforms that bind to the T-cell receptor and facilitate antigen recognition. These changes are characteristic of cells that have been activated to become armed effector T cells, yet some of the cells on which these changes have occurred have many characteristics of resting CD4 T cells, suggesting that they represent memory CD4 T cells. Only after re-exposure to antigen on a professional antigen-presenting cell do they achieve armed effector T-cell status, and acquire all the characteristics of armed T_H2 or T_H1 cells, secreting IL-4 and IL-5, or IFN-γ and TNF-β, respectively. As with memory CD8 T cells, the field will soon be revolutionized by direct staining of CD4 T cells with peptide:MHC class II dimers or tetramers.

It thus seems reasonable to designate these cells as memory CD4 T cells. Together these observations suggest that naive CD4 T cells can differentiate into armed effector T cells or into memory T cells; whether armed effector T cells can persist *in vivo*, and whether they can differentiate into memory T cells, is not yet clear.

Fig. 10.37 Many cell-surface molecules alter their expression on memory T cells. This is seen most clearly with CD45, where there is a change in the isoforms expressed (see Fig. 10.38). Many of these changes are also seen on cells that have been activated to become armed effector T cells. The changes increase the adhesion of the T cell to antigen-presenting cells and to endothelial cells. They also increase the sensitivity of the memory T cell to antigen stimulation.

Molecule	Other names	Relative expression on cells of indicated subset		Comments
		Naive	Memory	
LFA-3	CD58	1	>8	Ligand for CD2, involved in adhesion and signaling
CD2	T11	1	3	Mediates T-cell adhesion and activation
LFA-1	CD11a/ CD18	1	3	Mediates leukocyte adhesion and signaling
α_4 integrin	VLA4	1	4	Involved in T-cell homing to tissues
CD44	Ly24 Pgp-1	1	2	Lymphocyte homing to tissues
CD45RO		1	30	Lowest molecular weight isoform of CD45
CD45RA		10	1	High molecular weight isoform of CD45
L-selectin		High	Most low, some high	Lymph node homing receptor
CD3		1.0	1.0	Part of antigen-specific receptor complex

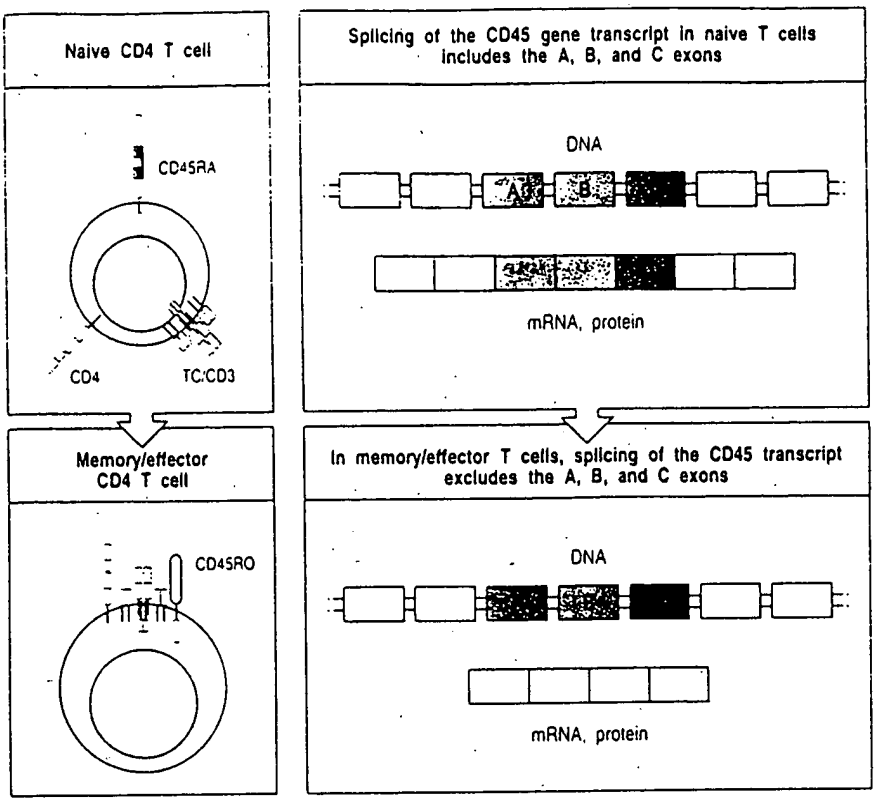


Fig. 10.38 Memory CD4 T cells express altered CD45 isoforms that regulate the interaction of the T-cell receptor with its co-receptors. CD45 is a transmembrane tyrosine phosphatase with three variable exons (A, B, and C) that encode part of its external domain. In naive T cells, high molecular weight isoforms (CD45RA) are found that do not associate with either the T-cell receptor (TC/CD3) or co-receptors (CD4). In memory T cells, the variable exons are removed by alternative splicing of CD45 RNA, and this isoform, known as CD45RO, associates with both the T-cell receptor and the co-receptor. This receptor complex seems to transduce signals more effectively than the receptor on naive T cells.

10-28 Retained antigen might have a role in immunological memory.

A successful adaptive immune response clears antigen from the body, halting further activation of naive lymphocytes. Antibody levels gradually decline, and effector T cells can no longer be detected. Residual antigen is difficult to detect, either in the form of surviving infectious agents or as antigens derived from them. Nevertheless, some antigen is probably retained for long periods as immune complexes bound to follicular dendritic cells in lymphoid follicles (see Fig. 10.34). Some intact virions might persist in this site as well, and a few infected cells might escape immune elimination. It has been proposed that this residual antigen is crucial for sustaining the cells that mediate immunological memory.

The long-lived cells that mediate immunological memory might be derived from activated naive T cells that differentiate directly into memory T cells, or they might first differentiate into effector T cells, which then either become long-lived memory T cells or are short-lived and undergo apoptosis (Fig. 10.39). Antigen has a critical role in determining the fate of the activated T cells, in a fashion reminiscent of positive selection in the thymus (see Chapter 7). Thus, high doses of antigen can trigger apoptosis of the effector T cells, in

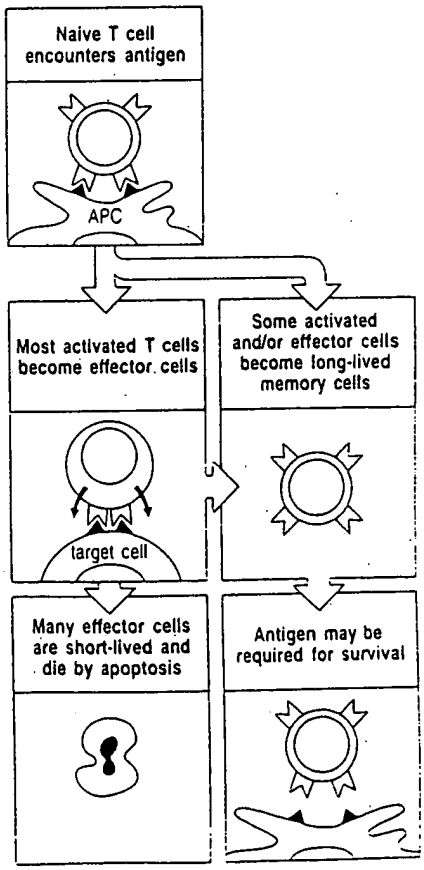


Fig. 10.39 Encounter with antigen generates effector T cells and long-lived memory T cells. Most of the effector T cells that are derived from antigen-stimulated naive T cells are relatively short lived, dying either from antigen overload or the absence of antigenic stimulus or sustaining cytokines. Some become long-lived memory T cells, which can also differentiate directly from armed effector T cells; antigenic stimulation might be required for these cells to persist. APC, antigen presenting cell.

much the same way as happens in clonal deletion; however, the absence of antigen can also lead to their apoptosis, just as developing T cells die if they are not positively selected. Memory T cells persist either because antigen has programmed them for a longer lifespan, because a low level of residual antigen preserves them by repetitive subthreshold signaling, or because the process that allows naive T cells to survive in the periphery acts more effectively on memory T cells.

It has proved difficult to determine experimentally whether antigen is absolutely required for the persistence of immunological memory. However, persistent antigen can clearly help to maintain a population of lymphocytes able to respond rapidly to the priming antigen. Thus, antigen retention in specialized sites, such as on follicular dendritic cells, where antigen persists for months or years (see Section 10-21), might be very important in immunological memory.

10-29 In immune individuals, secondary and subsequent responses are mediated solely by memory lymphocytes and not by naive lymphocytes.

In the normal course of an infection, a pathogen first proliferates to a level sufficient to elicit an adaptive immune response and then stimulates the production of antibodies and effector T cells that eliminate the pathogen from the body. Most of the armed effector T cells subsequently die and antibody levels gradually decline after the pathogen is eliminated, because the antigens that elicited the response are no longer present at the level needed to sustain it; we can think of this as feedback inhibition of the response. However, memory T and B cells remain, and maintain a heightened ability to mount a response to a recurrence of the infection.

The antibody and effector T cells remaining in an immunized individual also prevent the activation of naive B and T cells by the same antigen. Such a response would be wasteful, given the presence of memory cells that can respond much more quickly. The suppression of naive lymphocyte activation can be shown by passively transferring antibody or effector T cells to naive recipients; when the recipient is then immunized, naive lymphocytes do not respond to the original antigen but responses to other antigens are unaffected. This has been put to practical use to prevent the response of Rh^- mothers to their Rh^+ children (see Section 2-9); if anti-Rh antibody is given to the mother before she reacts to her child's red blood cells, her response will be inhibited. The mechanism of this suppression is known to involve the

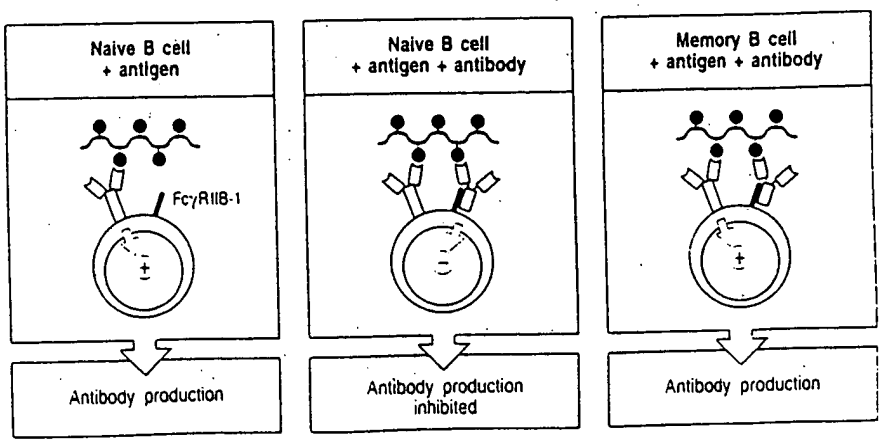


Fig. 10.40 Antibody can suppress naive B-cell activation by crosslinking the B-cell antigen receptor to the receptor FcγRIIB-1. Antigen binding to the B-cell antigen receptor delivers an activating signal (left panel); simultaneous signaling via the antigen receptor and FcγRIIB-1 delivers a negative signal to naive B cells (middle panel). Such cross-linking does not seem to affect memory B cells (right panel). This mechanism might have a role in suppressing naive B-cell responses in already primed individuals.

crosslinking of the B-cell antigen receptor to the isoform of FcγRII on the B-cell surface (FcγRIIB-1). Soluble antibody bound to antigen is able to link these two structures, which inhibits the activation of naive B cells (Fig. 10.40). FcγRIIB-1 has, in its intracellular domain, an immunoreceptor tyrosine-based inhibitory motif (ITIM) that inactivates signaling via the B-cell antigen receptor through a mechanism described in Section 5-14. For some reason, memory B-cell responses are not inhibited by antibody against the antigen, so the Rh^- mothers at risk must be identified and treated before a response has occurred. The ability of memory B cells to be activated to produce antibody even when exposed to pre-existing antibody allows secondary antibody responses to occur in individuals who are already immune.

Adoptive transfer of immune T cells to naive syngeneic mice also prevents the activation of naive T cells by antigen. This has been shown most clearly for cytotoxic T cells. It is possible that these memory CD8 T cells are activated to regain cytotoxic activity sufficiently rapidly that they can kill the antigen-presenting cells that are required to activate naive CD8 T cells, thereby inhibiting their activation.

These mechanisms might also explain the phenomenon known as **original antigenic sin**. This term was coined to describe the tendency of people to make antibodies only to epitopes expressed on the first influenza virus variant to which they were exposed, even in subsequent infections with variants that bear additional, highly immunogenic, epitopes (Fig. 10.41). Antibodies against the original virus will tend to suppress responses of naive B cells specific for the new epitopes by crosslinking their antigen receptors to FcγRIIB-1. This might benefit the host by using only those B cells that can respond most rapidly and effectively to the virus. This pattern is broken only if the person is exposed to an influenza virus that lacks all epitopes seen in the original infection, as now no pre-existing antibodies bind the virus and naive B cells are able to respond.

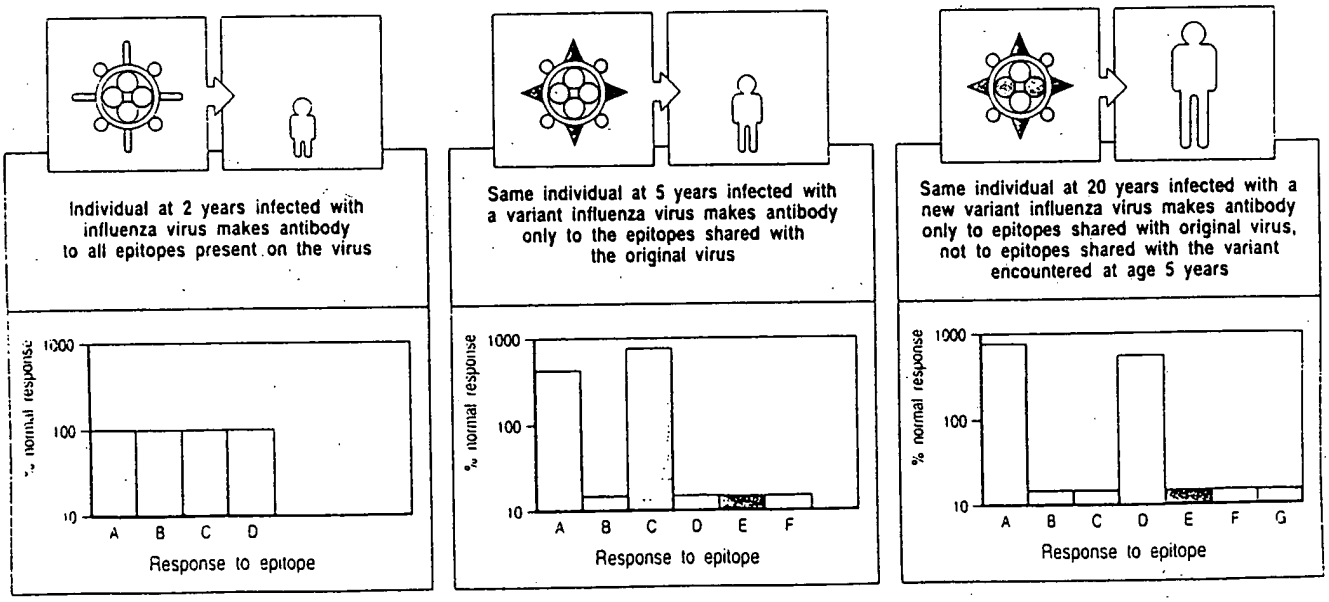


Fig. 10.41 Individuals who have already been infected with one variant of influenza virus make antibodies only to epitopes that were present on the initial virus variant when infected with a second variant. A child infected for the first time with an influenza virus makes a response to all epitopes (left panel). At age 5 years, the same child exposed to a variant virus responds preferentially to those epitopes shared with the original virus, and makes a smaller than normal response to new epitopes on the virus (middle panel). Even at age 20 years, this commitment to respond to epitopes shared with the original virus, and the subnormal response to new epitopes, is retained (right panel). This phenomenon is called 'original antigenic sin'.

Summary.

Protective immunity against re-infection is one of the most important consequences of adaptive immunity operating through clonal selection of lymphocytes. Protective immunity depends not only on pre-formed antibody and armed effector T cells, but mainly on immunological memory—an increased responsiveness to previously encountered pathogens that is long lived—and upon the establishment of a new population of memory lymphocytes. The capacity of these cells to respond rapidly to antigen can be transferred to naive recipients with primed B and T cells. The precise changes that distinguish naive, effector, and memory lymphocytes are not well characterized and, in T cells, the relative contributions of clonal expansion and differentiation to the memory phenotype are not yet clear. Memory B cells, however, can be distinguished by changes in their immunoglobulin genes because of isotype switching and somatic hypermutation, and secondary and subsequent immune responses are characterized by antibodies with increasing affinity for the antigen. It seems probable that residual antigen or infection is important in sustaining memory lymphocytes in some infections, although it is clearly not essential.

Summary to Chapter 10.

Vertebrates resist infection by pathogenic microorganisms in several ways. First, innate defenses against infection exclude infectious agents or kill them on first contact. For those pathogens that establish an infection, several early, non-adaptive responses are crucial to control infections and hold them in check until an adaptive immune response can be generated. Adaptive immunity takes several days to develop, as T and B lymphocytes must encounter their

	Phases of the immune response		
	Immediate (0–4 hours)	Early (4–96 hours)	Late (after 96 hours)
	Non-specific Innate No memory No specific T cells	Non-specific + specific Inducible No memory No specific T cells	Specific Inducible Memory Specific T cells
Barrier functions	Skin, epithelia	Local inflammation (C5a) Local TNF-α	IgA antibody in luminal spaces IgE antibody on mast cells
Response to extracellular pathogens	Phagocytes Alternative complement pathway	Mannan-binding lectin C-reactive protein T-cell independent B-cell antibody plus complement	IgG antibody and Fc receptor-bearing cells IgG, IgM antibody + classical complement pathway
Response to intracellular bacteria	Macrophages	Activated NK-dependent macrophage activation IL-1, IL-6, TNF-α, IL-12	T-cell activation of macrophages by IFN-γ
Response to virus-infected cells	Natural killer (NK) cells	Interferon-α and -β IL-12-activated NK cells	Cytotoxic T cells IFN-γ

Fig. 10.42 The components of the three phases of the immune response involved in defense against different classes of microorganisms. There are striking similarities in the effector mechanisms at each phase of the response; the main change is in the recognition structures used.

specific antigen, proliferate, and differentiate into effector cells. T-cell dependent B-cell responses cannot be initiated until antigen-specific T cells have had a chance to proliferate and differentiate. The same final effector mechanisms are used in all three phases of immunity; only the recognition mechanism changes (Fig. 10.42). Once an adaptive immune response has occurred, the infection is usually controlled, the pathogen contained or eliminated, and a state of protective immunity ensues. This state consists of the presence of effector cells and molecules produced in the initial response, and immunological memory. Immunological memory is manifest as a heightened ability to respond to pathogens that have been encountered previously and successfully eliminated. It is a property of memory T and B lymphocytes, which can transfer immune memory to naive recipients. However, the precise mechanism of immunological memory, which is arguably the most crucial feature of adaptive immunity, remains obscure. The artificial induction of protective immunity, including immunological memory, by vaccines is the most outstanding accomplishment of immunology in the field of medicine. Understanding how this is accomplished still lags behind its practical success.

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12 Allergy and Hypersensitivity

Allergic reactions occur when an individual who has produced IgE antibody in response to an innocuous antigen, or **allergen**, subsequently encounters the same allergen. The allergen triggers the activation of IgE-binding mast cells in the exposed tissue, leading to a series of responses that are characteristic of **allergy**. As we learned in Chapter 9, there are circumstances in which IgE is involved in protective immunity, especially in response to parasitic worms, which are prevalent in underdeveloped countries. In more advanced countries, however, IgE responses to innocuous antigens predominate and allergy is one of the most prevalent diseases (Fig. 12.1). Allergic reactions to common environmental antigens affect up to half the population in North America and Europe and, although they are rarely life-threatening, cause much distress and lost time from school and work. Because of the medical importance of allergy in industrialized societies, much more is known about the pathophysiology of IgE-mediated responses than about the normal physiological role of IgE.

Fig. 12.1 IgE-m diated reactions to extrinsic antig ns. All IgE-mediated respons s involv mast-cell degranulation, but the symptoms experienced by the patient can be very different depending on whether the allergen is injected, inhaled, or eaten, and depending also on the dose of the allergen.

IgE-mediated allergic reactions

Syndrome	Common allergens	Route of entry	Response
Systemic anaphylaxis	Drugs Serum Venoms Peanuts	Intravenous (either directly or following rapid absorption)	Edema Increased vascular permeability Tracheal occlusion Circulatory collapse Death
Acute urticaria (wheal-and-flare)	Insect bites Allergy testing	Subcutaneous	Local increase in blood flow and vascular permeability
Allergic rhinitis (hay fever)	Pollens (ragweed, timothy, birch) Dust-mite feces	Inhaled	Edema of nasal mucosa Irritation of nasal mucosa
Asthma	Pollens Dust-mite feces	Inhaled	Bronchial constriction Increased mucus production Airway inflammation
Food allergy	Shellfish Milk Eggs Fish Wheat	Oral	Vomiting Diarrhea Pruritis (itching) Urticaria (hives) Anaphylaxis (rarely)

The term allergy was originally defined by Clemens Von Pirquet as 'an altered capacity of the body to react to a foreign substance,' which was an extremely broad definition that included all immunological reactions. Allergy is now defined in a much more restricted manner as 'disease following a response by the immune system to an otherwise innocuous antigen.' Allergy is one of a class of immune system responses that are termed **hypersensitivity reactions**; these are harmful immune responses that produce tissue injury and may cause serious disease. Hypersensitivity reactions were classified into four types by Coombs and Gell (Fig. 12.2). Allergy is often equated with type I hypersensitivity (immediate-type hypersensitivity reactions mediated by IgE), and will be used in this sense here.

In this chapter we shall first consider the mechanisms that favor the production of IgE. We shall then describe the pathophysiological consequences of the interaction between antigen and IgE that is bound by the high-affinity Fcε receptor (FcεRI) on mast cells. Finally, we shall consider the causes and consequences of other types of immunological hypersensitivity reactions.

The production of IgE.

IgE differs from other antibody isotypes in being located predominantly in tissues, where it is bound to mast cells by high-affinity surface receptors called FcεRI. Binding of antigen to IgE crosslinks these receptors and this causes the release of mediators from the mast cells, which may lead to the development of a **type I hypersensitivity reaction**. Basophils and activated eosinophils also express FcεRI; they can therefore display surface-bound IgE and also take part

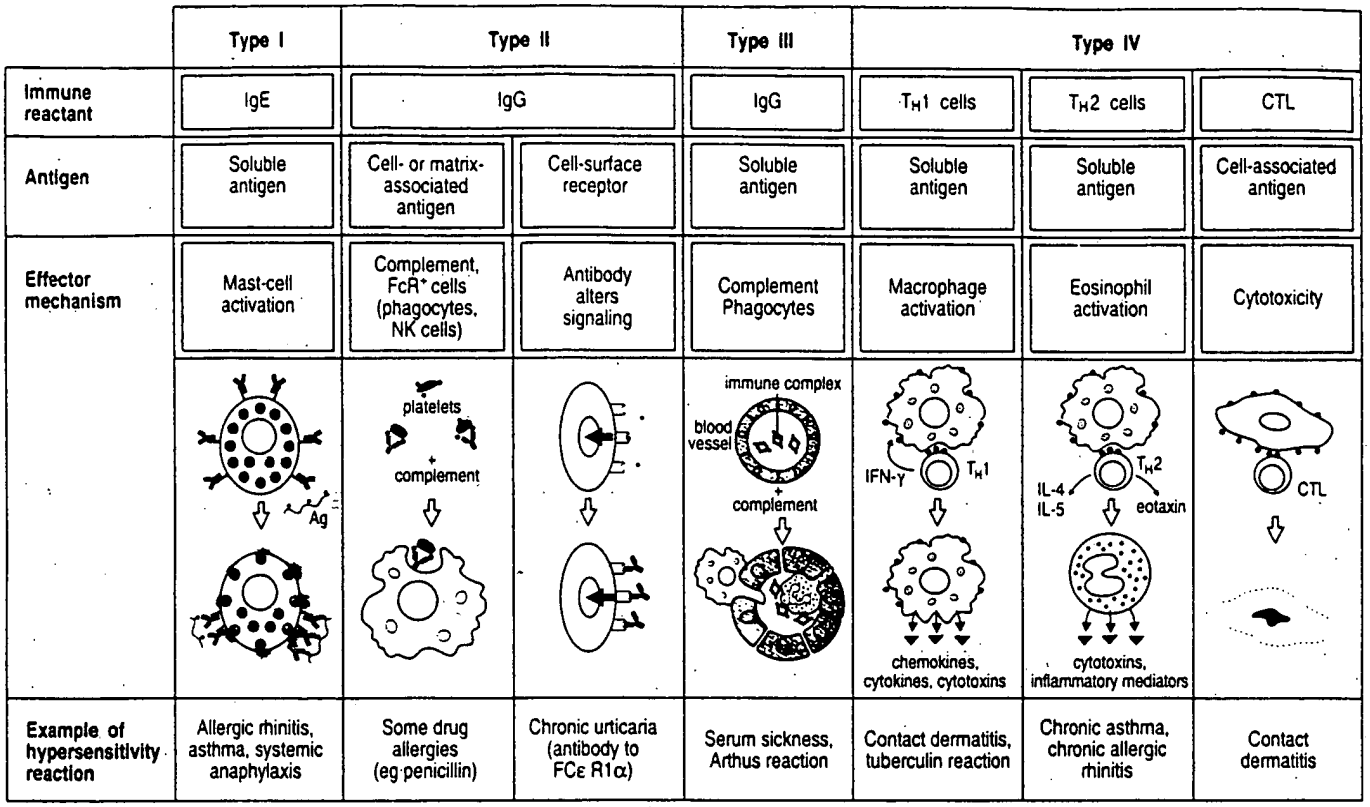

Fig. 12.2 There are four types of hypersensitivity reaction mediated by immunological mechanisms that tissue damage. Types I–III are antibody-mediated and are distinguished by the different types of antigens recognized and the different classes of antibody involved. Type I responses are mediated by IgE, which induces mast-cell activation, whereas types II and III are mediated by IgG, which can engage complement-mediated and phagocytic effector mechanisms to varying degrees, depending on the subclass of IgG and the nature of the antigen involved. Type II responses are directed against cell-surface or matrix antigens, whereas type III responses are directed against soluble antigens, and the tissue damage involved is caused by responses triggered by immune complexes. A special category of type II responses involves IgG antibodies against cell-surface receptors that disrupt the normal functions of the receptor, either by causing uncontrollable activation or by blocking receptor function. Type IV hypersensitivity reactions are T-cell mediated and can be subdivided into three groups. In the first group, tissue damage is caused by the activation of macrophages by T_H1 cells, which results in an inflammatory response. In the second, damage is caused by the activation of eosinophilic inflammatory responses by T_H2 cells; in the third, damage is caused directly by cytotoxic T cells (CTL).

in the production of type I hypersensitivity reactions. The factors that lead to an antibody response dominated by IgE are still being worked out. Here we shall describe our current understanding of these processes before turning to the question of how IgE mediates allergic reactions.

12-1 Allergens are often delivered transmucosally at low dose, a route that favors IgE production.

There are certain antigens and routes of antigen presentation to the immune system that favor the production of IgE. As we learned in Chapter 9, T_H2 cells can switch the antibody isotype from IgM to IgE, or they can cause switching to IgG2 and IgG4 (human) or IgG1 and IgG3 (mouse). Antigens that selectively evoke T_H2 cells that drive an IgE response are known as allergens.

Much human allergy is caused by a limited number of inhaled small protein allergens that reproducibly elicit IgE production in susceptible individuals. Because we inhale many different proteins that do not induce IgE production,

Features of inhaled allergens that may promote the priming of T_H2 cells that drive IgE responses	
Protein	Only proteins induce T-cell responses
Enzymatically active	Allergens are often proteases
Low dose	Favors activation of IL-4-producing CD4 T cells
Low molecular weight	Diffuses out of particle into mucus
High solubility	Readily eluted from particle
Stable	Allows survival in desiccated particle
Contains peptides that bind host MHC class II	Required for T-cell priming

Fig. 12.3 Properties of inhaled allergens. The typical characteristics of inhaled allergens are described in this table.

what is unusual about the proteins that are common allergens? Although we do not yet have a complete answer, some general principles have emerged (Fig. 12.3). Most allergens are relatively small, highly soluble, proteins that are carried on desiccated particles such as pollen grains or mite feces. On contact with the mucosa of the airways, for example, the soluble allergen elutes from the particle and diffuses into the mucosa. Allergens are typically presented to the immune system at very low doses. It has been estimated that the maximum exposure of a person to the common pollen allergens in ragweed (*Artemisia artemisiifolia*) does not exceed 1 μg per year! Yet many people develop irritating and even life-threatening T_H2-driven IgE antibody responses to these minute doses of allergen. It is important to note that only some of the people who are exposed to these substances make IgE antibodies against them. Possible factors that influence which individuals will respond to allergens are considered in Section 12-4.

It seems likely that presenting an antigen transmucosally and at very low doses is a particularly efficient way of inducing T_H2-driven IgE responses. IgE antibody production requires IL-4-producing T_H2 cells and it can be inhibited by T_H1 cells that produce interferon-γ (IFN-γ) (see Fig. 9.8). We have already learned that the presentation of low doses of antigen can favor the activation of T_H2 cells over T_H1 cells (see Section 10-19), and many common allergens are delivered to the respiratory mucosa by inhalation of a low dose. The dominant antigen-presenting cell type in the respiratory mucosa is a cell with characteristics similar to those of Langerhans' cells. These cells very efficiently take up and process protein antigens, a step that is accompanied by cellular activation. This in turn induces their migration to regional lymph nodes and differentiation into cells that are highly co-stimulatory, with characteristics that favor T_H2 differentiation.

12-2 Enzymes are frequent triggers of allergy.

In Chapter 9, we learned that several lines of evidence suggest that IgE is important in host defense against parasites. Many parasites invade their hosts by secreting proteolytic enzymes that break down connective tissue and allow the parasite access to host tissues, and it has been proposed that these enzymes are particularly active at promoting T_H2 responses. This idea receives some support from the many examples of allergens that are enzymes. The major allergen of the house dust mite (*Dermatophagoides pteronyssimus*) which is responsible for allergy in up to 20% of the North American population, is a cysteine protease homologous to papain. Papain itself, derived from the papaya fruit, is used as a meat tenderizer and causes allergy in workers preparing the enzyme; such allergies are called industrial allergies. Another industrial allergy is the asthma caused by inhalation of the bacterial enzyme subtilisin, the 'biological' component of some laundry detergents.

Injection of enzymatically active papain (but not inactivated papain) into mice stimulates an IgE response. A closely related enzyme, chymopapain, is used medically to destroy intervertebral disks in patients with sciatica; the major (although rare) complication of this procedure is anaphylaxis, an acute systemic response to allergens (see Section 12-11). Not all allergens are enzymes, however; for example, two allergens identified from filarial worms are enzyme inhibitors. Many protein allergens derived from plants have been identified and sequenced, but their functions are currently obscure. Thus, the association between enzymatic activity and allergenicity is intriguing but of unproven importance.

12-3 Class switching to IgE in B lymphocytes is favored by specific accessory signals.

IgE production requires cytokines that are released by T_H2 cells, in particular interleukin (IL)-4. T_H2 cells arise when naive CD4 T cells first encounter antigen in the presence of IL-4. The importance of IL-4 in driving IgE production is seen in mice lacking a functional IL-4 gene: the major abnormality in these mice seems to be reduced IgE synthesis. In mice, the early production of IL-4 has been shown to be the result of activation of a small subset of CD4 T cells with unusual properties. These cells, the NK1.1$^+$ subset, express T-cell receptors made up of a restricted set of β chains and an invariant α chain, and develop in response to CD1, an MHC class I-like molecule found in humans as well as mice (Fig. 12.4). Evidence for their development in response to CD1 derives from the absence of NK1.1$^+$ T cells in mice that cannot express CD1 molecules because of engineered defects in their β_2-microglobulin genes. The invariant T-cell receptor α chain expressed by NK1.1$^+$ T cells is encoded in a single V_α gene segment and a single J_α gene segment; similar cells in humans are also specific for the human homolog of mouse CD1, called CD1d, and use the homologous V_α and J_α gene segments. These T cells produce IL-4 almost immediately upon encountering CD1, which is present on cortical thymocytes and on Langerhans' cells and other antigen-presenting cells. In mice, CD1-specific T cells are the only known source of early IL-4; mice lacking β_2-microglobulin fail to make early IL-4 and are deficient in IgE production. It has not yet been established whether this pathway is important in humans.

As noted above, IL-4 drives naive CD4 T cells to differentiate into T_H2 cells and inhibits T_H1 development. Once T_H2 cells are primed, they can deliver several molecular signals that favor class switching in B lymphocytes to IgE (see Section 9-5); for example, when their T-cell receptors are ligated, they secrete cytokines such as IL-4 and IL-13. Activated T_H2 cells also express CD40 ligand (CD40L) and CD23 (the low-affinity receptor for IgE), and these co-stimulatory molecules then ligate their counter-receptors CD40 and CR2 on B lymphocytes. The combination of these signals causes class switching to IgE and B-cell proliferation.

The IgE response, once initiated, can be further amplified by basophils, mast cells, and eosinophils, which can also drive IgE production (Fig. 12.5). All three cell types express FcεRI, although eosinophils only express it when activated. When these specialized granulocytes are activated by antigen crosslinking of their FcεRI-bound IgE, they can express cell-surface CD40L

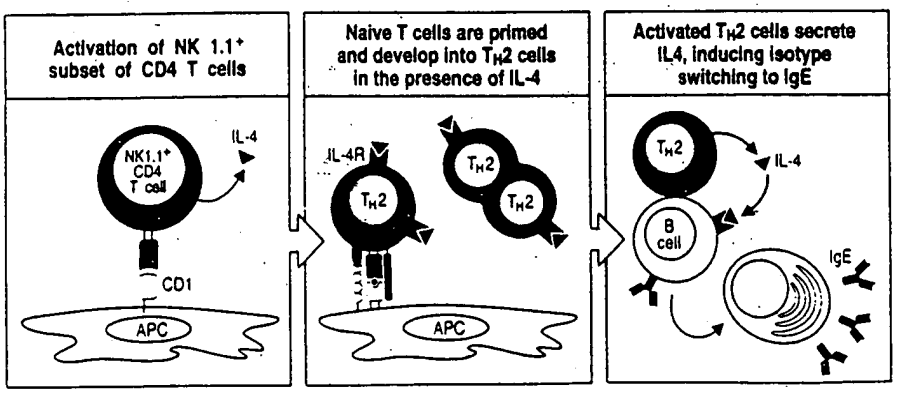

Fig. 12.4 IgE class switching in B cells is initiated by T_H2 cells, which develop in the presence of an early burst of IL-4. IL-4 is secreted early in some immune responses by a small subset of CD4 T cells (NK1.1$^+$ CD4 T cells) which interact with antigen-presenting cells bearing the non-classical MHC class I-like molecule CD1. Naive T cells being primed by their first encounter with antigen are driven to differentiate into T_H2 cells in the presence of this early burst of IL-4. These mechanisms have been characterized in mice; it is not yet known whether the same pathways operate in humans.

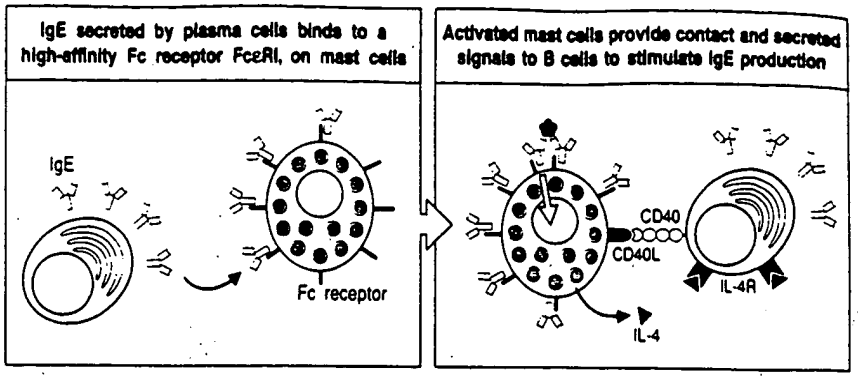

Fig. 12.5 Antig n ligati n of IgE bound to mast cells leads t amplification f IgE producti n. IgE secreted by plasma cells binds to the high-affinity IgE receptor on mast cells, basophils, and activated eosinophils. When the surface-bound IgE is crosslinked by antigen these cells express CD40L and secrete IL-4, which in turn stimulates isotype switching by B cells and the production of more IgE. These interactions can occur *in vivo* at the site of allergen-triggered inflammation, for example in bronchial-associated lymphoid aggregates.

and secrete IL-4; like T_H2 cells, therefore, they can drive class switching and IgE production by B cells. The interaction between these specialized granulocytes and B cells can occur at the site of the allergic reaction, as B cells are observed to form germinal centers at inflammatory foci. Blocking this amplification process is a goal of therapy, as allergic reactions can otherwise become self sustaining.

12-4 Genetic factors contribute to the development of IgE-mediated allergy, but environmental factors may also be important.

Up to 40% of people in Western populations show an exaggerated tendency to mount IgE responses to a wide variety of common environmental allergens. This state is called **atopy** and seems to be influenced by several genetic loci. Atopic individuals have higher total levels of IgE in the circulation and higher levels of eosinophil than their normal counterparts. They are more susceptible to allergic diseases such as hay fever and asthma. Studies of atopic families have implicated loci on chromosomes 11q and 5q that might be important in determining the presence of atopy, and candidate genes that might affect IgE responses are found in these regions. The candidate gene on chromosome 11 encodes the β subunit of the high-affinity IgE receptor, whereas on chromosome 5 there is a cluster of tightly linked genes that includes those for IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. These cytokines are important in IgE isotype switching, eosinophil survival, and mast-cell proliferation. Of particular note, an inherited genetic variation in the promoter region of the IL-4 gene is associated with raised IgE levels in atopic individuals; the variant promoter will direct increased expression of a reporter gene in experimental systems. It is too early to know whether this polymorphism is important in the complex genetics of atopy.

A second type of inherited variation in IgE responses is linked to the MHC class II region and affects responses to specific allergens. Many studies have shown that specific IgE production to individual allergens is associated with particular HLA class II alleles, implying that particular MHC:peptide combinations might favor a strong T_H2 response. For example, IgE responses to several ragweed pollen allergens are particularly associated with haplotypes containing the MHC class II allele, DRB1*1501. Many individuals are therefore generally predisposed to make T_H2 responses and specifically predisposed to respond to some allergens more than others. However, allergies to common drugs such as penicillin show no association with MHC class II or the presence or absence of atopy.

The prevalence of atopic allergy and, in particular, of asthma is increasing in economically advanced regions of the world, an observation that is best explained by environmental factors. Three candidate environmental factors for which there is little supporting evidence are changes in allergen levels, environmental pollution, and dietary changes. Alterations in exposure to microbial pathogens is the most plausible explanation at present for the increase in atopic allergy. Atopy is negatively associated with a history of infection by measles or hepatitis A virus, and with positive tuberculin skin tests (suggesting prior exposure and immune response to *Mycobacterium tuberculosis*). It is possible that infection by an organism that evokes a T_H1 immune response early in life might reduce the likelihood of T_H2 responses later in life.

Summary.

Allergic reactions are the result of the production of specific IgE antibody to common, innocuous antigens. Allergens are small antigens that commonly provoke an IgE antibody response. Such antigens normally enter the body at very low doses by diffusion across mucosal surfaces and trigger a T_H2 response. Naive allergen-specific T cells are induced to develop into T_H2 cells in the presence of an early burst of IL-4, which seems to be derived from a specialized subset of T cells. The allergen-specific T_H2 cells drive allergen-specific B cells to produce IgE, which binds to the high-affinity receptor for IgE on mast cells, basophils, and activated eosinophils. IgE production can be amplified by these cells because, upon activation, they produce IL-4 and CD40L. The tendency to the production of IgE is influenced by genetic and environmental factors. Once IgE is produced in response to an allergen, re-exposure to the allergen triggers an allergic response by mechanisms to which we now turn.

Effector mechanisms in allergic reactions.

Allergic reactions are triggered when allergens crosslink preformed IgE bound to the high-affinity receptor FcεRI on mast cells. Mast cells line the body surfaces and serve to alert the immune system to local infection. In allergy, they provoke very unpleasant reactions to innocuous antigens that are not associated with invading pathogens that need to be expelled. Mast cells act by releasing stored mediators by granule exocytosis, and also by synthesizing leukotrienes and cytokines (see Fig. 12.11). The consequences of IgE-mediated mast-cell activation depend on the dose of antigen and its route of entry; symptoms range from the irritating sniffles of hay fever when pollen is inhaled, to the life-threatening circulatory collapse that occurs in systemic anaphylaxis (Fig. 12.6). The immediate allergic reaction caused by mast-cell degranulation is followed by a more sustained inflammation, known as the late-phase response. This late response involves the recruitment of other effector cells, notably T_H2 lymphocytes, eosinophils, and basophils, which contribute significantly to the immunopathology of an allergic response.

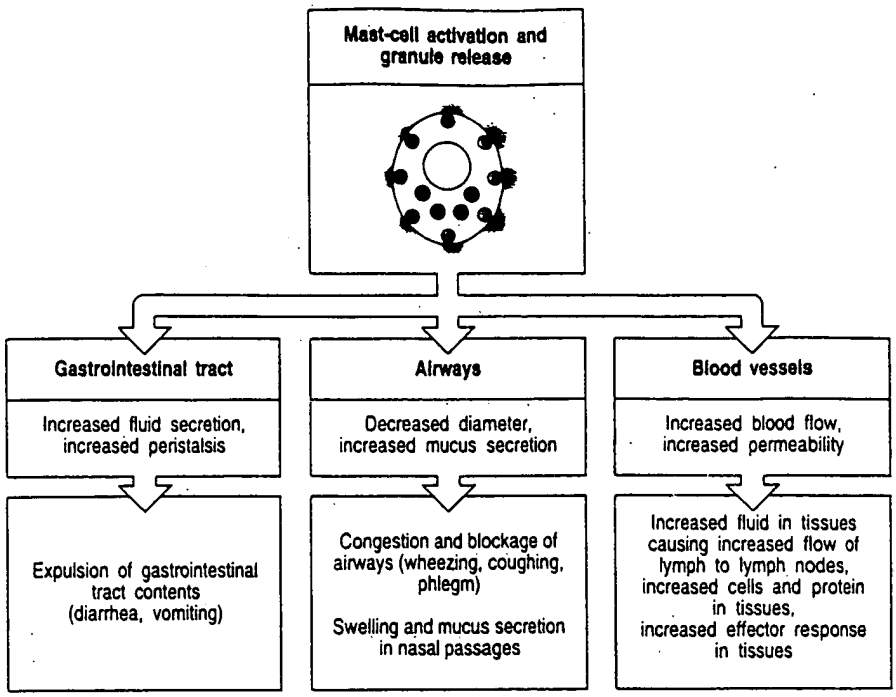

Fig. 12.6 Mast-cell products have different eff cts on differ nt tissues. Mast-cell products can be divided into two categories: first, those molecules, both preformed and rapidly synthesized, that mediate acute inflammatory events after mast-cell activation; and second, cytokines and lipid mediators, which induce a late-phase chronic inflammatory response with influx and activation of T_H2 lymphocytes, monocytes, eosinophils, and neutrophils. There is some overlap between mediators that induce acute and chronic inflammatory responses, particularly among the lipid mediators, which have rapid effects causing smooth muscle contraction, increased vascular permeability, and mucus secretion, and also induce the influx and activation of leukocytes, which contribute to the late-phase response.

12-5 Most IgE is cell-bound and engages effector mechanisms of the immune system by different pathways from other antibody isotypes.

Most antibodies are found in body fluids and engage effector cells (through receptors specific for the Fc constant regions) only after binding specific antigen (through their variable regions). IgE is an exception, however, as it is captured by high-affinity receptors specific for the IgE Fc region in the absence of bound antigen. This means that IgE is mostly found fixed in the tissues on mast cells that bear this receptor, as well as on circulating basophils and activated eosinophils. The ligation of cell-bound IgE by antigen triggers activation of these cells at the site of antigen entry into the tissues. The release of inflammatory lipid mediators, cytokines, and chemokines at sites of IgE-triggered reactions results in the recruitment of eosinophils and basophils to augment the type I response.

There are two types of IgE-binding Fc receptor. The first, FcεRI, is a high-affinity receptor of the immunoglobulin superfamily that binds IgE on mast cells, basophils, and activated eosinophils (see Chapter 9). When the cell-bound IgE is crosslinked, FcεRI transduces an activating signal. High levels of IgE, such as those that exist in subjects with allergic diseases or parasite infections, can result in marked increases in surface FcεRI expression on mast cells, enhanced sensitivity of such cells to activation by low concentrations of specific antigen, and markedly increased IgE-dependent release of mediators and cytokines. The second IgE receptor, CD23, is a structurally unrelated molecule that binds IgE with low affinity. CD23 is found on many different cell types, including B cells, activated T cells, monocytes, eosinophils, platelets, follicular dendritic cells, and some thymic epithelial cells. This receptor was thought to be crucial for the regulation of IgE antibody levels; however, a mouse strain in which the CD23 gene was deleted by homologous recombination (see Section 2-27) shows no major abnormality in the development of polyclonal IgE responses. These mice did not show antigen-

specific IgE-mediated enhancement of antibody responses, however. This demonstrates a role for CD23 on antigen-presenting cells in the capture of antigen by specific IgE.

12-6 Mast cells reside in tissues and orchestrate allergic reactions.

Mast cells were described by Ehrlich in the mesentery of rabbits and named *Mastzellen* ('fattened cells'). Like basophils, mast cells contain granules rich in acidic molecules that take up basic dyes. However, in spite of this resemblance, and the similar range of mediators stored in these basophilic granules, mast cells are derived from a different myeloid lineage from basophils and eosinophils. Mast cells are highly specialized cells, and are prominent residents of mucosal and epithelial tissues in the vicinity of small blood vessels and postcapillary venules, where they are well placed to guard against invading pathogens (see Sections 9-20 and 9-21). Mast cells are also found in subendothelial connective tissue. They home to tissues as agranular cells; their final differentiation, accompanied by granule formation, occurs after they have arrived in the tissues. The major mast-cell growth factor is stem-cell factor (SCF), whose receptor, c-Kit (CD117), is encoded by a proto-oncogene. Mice with defective c-Kit lack differentiated mast cells and studies of these mice have shown that IgE-mediated inflammatory responses are dependent almost exclusively on mast cells.

Mast cells express FcεRI constitutively on their surface and they are activated when antigens crosslink FcεRI-bound IgE. Degranulation occurs within seconds, releasing a variety of preformed mediators (see Figs 12.11 and 9.30). Among these are histamine—a short-lived vasoactive amine that causes an immediate increase in local blood flow and vessel permeability—and the enzymes mast-cell chymase, tryptase, and serine esterases. The latter might in turn activate matrix metalloproteinases, which collectively break down tissue matrix proteins. Tumor necrosis factor (TNF)-α is also stored in mast-cell granules and is released in large amounts from both preformed and newly synthesized pools on mast-cell activation. It causes endothelial activation with upregulation of the expression of adhesion molecules, which promotes the influx of inflammatory leukocytes and lymphocytes.

On mast-cell activation, chemokines, lipid mediators such as leukotrienes and platelet-activating factor (PAF), and further cytokines such as IL-4, are synthesized and act to sustain the inflammatory response. Thus, the IgE-mediated activation of mast cells orchestrates an important inflammatory cascade that is amplified by the recruitment of eosinophils, basophils, and T_H2 lymphocytes. The physiological importance of this is as a host defense mechanism, as we learned in Chapter 9. In allergy, however, the acute and chronic inflammatory reactions triggered by mast-cell activation can also have important pathophysiological consequences, as seen in the diseases associated with allergic responses to environmental antigens.

12-7 Eosinophils are normally under tight control to prevent inappropriate toxic responses.

Eosinophils are bone marrow-derived granulocytic leukocytes, so named because their granules, which contain arginine-rich basic proteins, are colored bright orange by the acidic stain eosin (Fig. 12.7). Only very small numbers of these cells are normally present in the circulation; most eosinophils are found

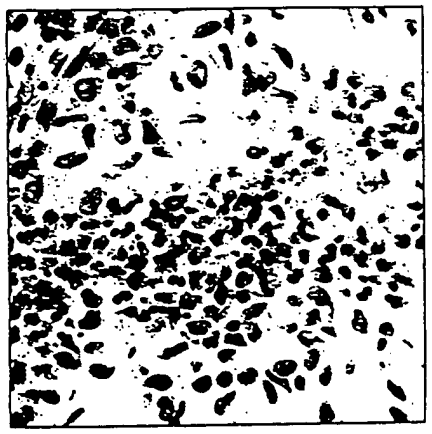

Fig. 12.7 Eosinophils can be detected easily in tissue sections by their bright orange coloration. In this light micrograph, a large number of eosinophils are seen infiltrating a Langerhans' cell histiocytosis. The tissue section is stained with hematoxylin and eosin; it is the eosin that imparts th characteristic orang color to the eosinophils. Photograph courtesy of T Krausz.

in tissues, especially in the connective tissue immediately underneath respiratory, gut, and urogenital epithelium, implying a likely role for these cells in defense against invading organisms. Eosinophils have two kinds of effector function. First, they release highly toxic granule proteins and free radicals, which can kill microorganisms and parasites but can also cause significant tissue damage in allergic reactions. Second, they produce molecules including prostaglandins, leukotrienes, and cytokines, which amplify the inflammatory response by recruiting and activating further eosinophils, leukocytes, and epithelial cells (Fig. 12.8).

Important regulatory mechanisms inhibit the inappropriate activation and degranulation of eosinophils, which could otherwise be very harmful to the host. The first level of control regulates the production of eosinophils by the bone marrow, which is low in the absence of infection or other immune stimulation. When T_H2 cells are activated, cytokines such as IL-5 are released that increase the production of eosinophils in the bone marrow and promote their release into the circulation. However, transgenic animals overexpressing IL-5 show eosinophilia in the circulation but not in tissues. This demonstrates that a second level of control on eosinophil activity regulates the migration of eosinophils from the circulation into tissues. The key molecules in this response are CC chemokines (see Section 10-9). Most chemokines cause chemotaxis of several types of leukocyte; two of the newest members of the CC family are specific for eosinophils and have been named **eotaxin 1** and **eotaxin 2**.

The eotaxin receptor on eosinophils, CCR3, is a member of the chemokine family of receptors (see Section 10-9). As well as the eotaxins, this receptor also binds the chemokines MCP-3, MCP-4, and RANTES, providing an explanation for the finding that these chemokines can also induce eosinophil activation

Fig. 12.8 Eosinophils secrete a range of highly toxic granule proteins and other inflammatory mediators.

Class of product	Examples	Biological effects
Enzyme	Eosinophil peroxidase	Toxic to targets by catalyzing halogenation Triggers histamine release from mast cells
	Eosinophil collagenase	Remodeling of connective tissue matrix
Toxic protein	Major basic protein	Toxic to parasites and mammalian cells Triggers histamine release from mast cells
	Eosinophil cationic protein	Toxic to parasites Neurotoxin
	Eosinophil-derived neurotoxin	Neurotoxin
Cytokine	IL-3, IL-5, GM-CSF	Amplify eosinophil production by bone marrow Cause eosinophil activation
Chemokine	IL-8	Promotes influx of leukocytes
Lipid mediator	Leukotrienes C4 and D4	Smooth muscle contraction Increased vascular permeability Mucus secretion
	Platelet-activating factor	Chemotactic to leukocytes Amplifies production of lipid mediators Neutrophil, eosinophil, and platelet activation

and chemotaxis. T_H2 cells have also been found to carry CCR3, showing that, as well as cytokines, families of chemokine molecules can coordinate certain kinds of immune response.

The third level of eosinophil regulation is control of their state of activation. In their non-activated state, eosinophils do not express high-affinity IgE receptors and have a high threshold for release of their granule contents. After activation by cytokines and chemokines this threshold drops, FcεRI is expressed, and the numbers of surface complement and Fcγ receptors increase. The eosinophil is now primed to express effector activity.

The potential of eosinophils to cause tissue injury is illustrated by rare hypereosinophilic syndromes. These are sometimes seen in association with T-cell lymphomas in which unregulated IL-5 secretion drives a marked increase in the numbers of eosinophils in the blood (hypereosinophilia). The clinical manifestations of hypereosinophilia are damage to the endocardium (Fig. 12.9) and to nerves, leading to heart failure and neuropathy, both thought to be caused by the toxic effects of eosinophil granule proteins.

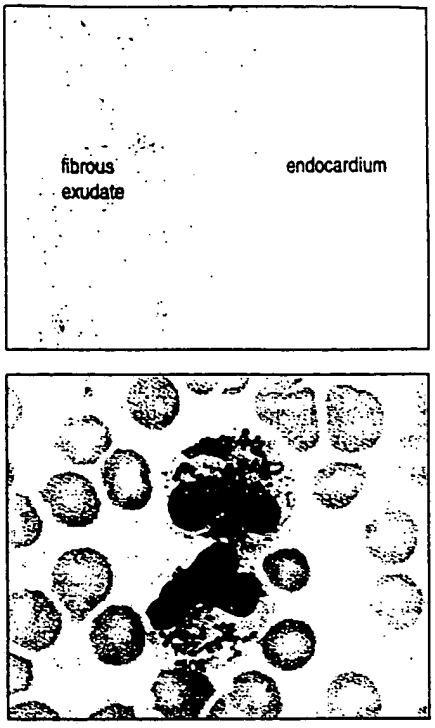

Fig. 12.9 Hypereosinophilia can cause injury to the endocardium. The top panel shows a section of the endocardium from a patient with hypereosinophilic syndrome. There is an organized fibrous exudate and the underlying endocardium is thickened by fibrous tissue. Although there are large numbers of circulating eosinophils, these cells are not seen in the injured endocardium, which is thought to be damaged by granules released from circulating eosinophils. The panel on the bottom shows two partly degranulated eosinophils (center) surrounded by erythrocytes in a peripheral blood film. Photographs courtesy of D Swirsky and T Krausz.

12-8 Eosinophils and basophils cause inflammation and tissue damage in allergic reactions.

In a local allergic reaction, mast-cell degranulation and T_H2 activation cause eosinophils to accumulate in large numbers and to become activated. Their continued presence is characteristic of chronic allergic inflammation and they are thought to be major contributors to the tissue damage that occurs.

Basophils are also present at the site of the reaction. These are bone marrow-derived granulocytes, which share a common stem-cell precursor with eosinophils. Growth factors for basophils are very similar to those for eosinophils and include IL-3, IL-5, and GM-CSF. There is evidence for reciprocal control of the maturation of the stem-cell population into basophils or eosinophils. For example, transforming growth factor (TGF)-β in the presence of IL-3 suppresses eosinophil differentiation and enhances that of basophils. Basophils are normally present in very low numbers in the circulation and seem to have a similar role to that of eosinophils in host defense against invading pathogens. Like eosinophils, they are recruited to the sites of allergic reactions. Basophils express FcεRI on the cell surface and, on activation, they release toxic mediators from the basophilic granules after which they are named.

Eosinophils, mast cells, and basophils can interact with each other. Eosinophil degranulation causes the release of **major basic protein**, which in turn causes mast cell and basophil degranulation. This effect is augmented by the presence of any of the cytokines that affect eosinophil and basophil growth, differentiation, and activation, such as IL-3, IL-5, and GM-CSF.

12-9 The allergic reaction after ligation of IgE on mast cells is divided into an immediate response and a late-phase response.

The inflammatory response after IgE-mediated mast-cell activation occurs as an immediate reaction, starting within seconds, and a late reaction, which takes up to 8–12 hours to develop. These reactions can be distinguished clinically (Fig. 12.10). The **immediate reaction** follows from the activity of histamine, prostaglandins, and other preformed or rapidly synthesized toxic mediators

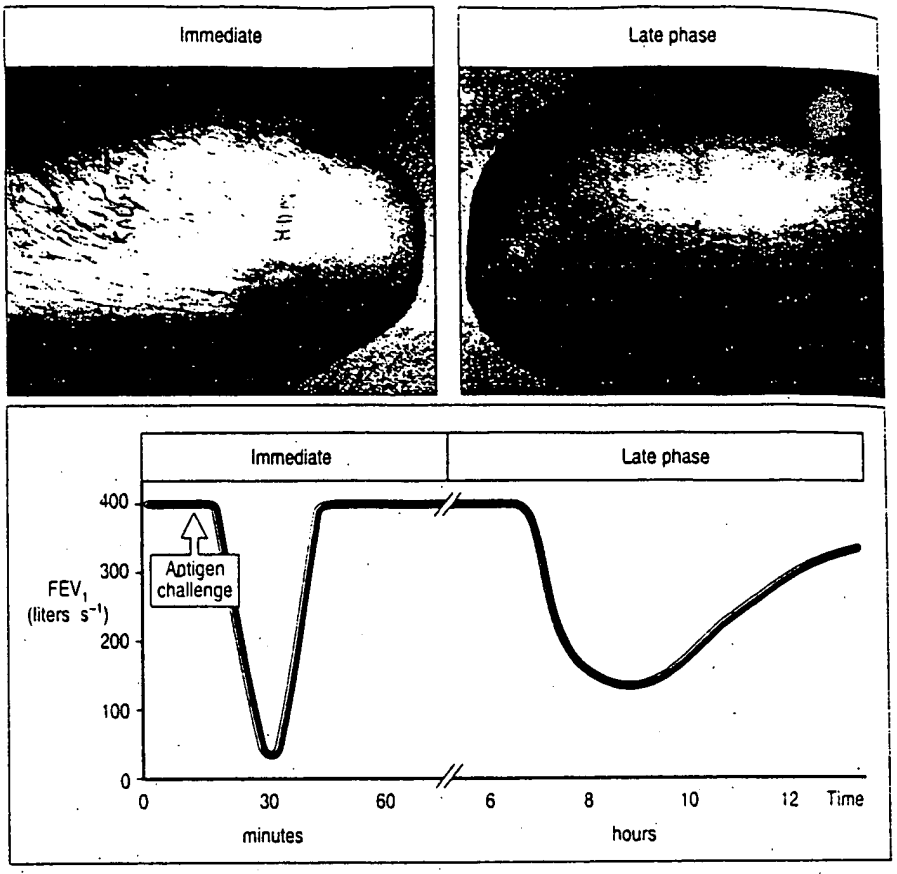

Fig. 12.10 Allergic reactions can be divided into an immediate response and a lat -phase response. A wheal-and-flare allergic reaction develops within a minute or two of superficial injection of antigen into the epidermis and lasts for up to 30 minutes. The reaction to an intracutaneous injection of house dust mite antigen is shown in the upper left panel and is labeled HDM: the area labeled saline shows the absence of any response to a control injection of saline solution. A more widespread edematous response, as shown in the upper right panel, develops approximately 8 hours later and can persist for some hours. Similarly, the response to an inhaled antigen can be divided into early and late responses (bottom panel). An asthmatic response in the lungs with narrowing of the airways caused by the constriction of bronchial smooth muscle can be measured as a fall in the forced expired volume of air in one second (FEV_1). The immediate response peaks within minutes after antigen inhalation and then subsides. Approximately 8 hours after antigen challenge, there is a late-phase response that also results in a fall in the FEV_1. The immediate response is caused by the direct effects on blood vessels and smooth muscle of rapidly metabolized mediators such as histamine released by mast cells. The late-phase response is caused by the effects of an influx of inflammatory leukocytes attracted by chemokines and other mediators released by mast cells during and after the immediate response. Photographs courtesy of A B Kay.

that cause a rapid increase in vascular permeability and the contraction of smooth muscle. The **late-phase reaction** is caused by the induced synthesis and release of mediators including leukotrienes, chemokines, and cytokines from the activated mast cells. These recruit leukocytes, including eosinophils and T_H2 lymphocytes, to the site. Although the late-phase reaction is clinically less marked than the immediate response, it is associated with a second phase of smooth muscle contraction and sustained edema. The molecules synthesized and released by mast cells after activation are listed in Fig. 12.11.

The late-phase reaction is an important cause of much more serious long-term illness, as, for example, in chronic asthma. This is because the late reaction induces the recruitment of inflammatory leukocytes, especially eosinophils and T_H2 lymphocytes, to the site of the allergen-triggered mast-cell response. This late response can easily convert into a chronic inflammatory response if antigen persists and stimulates allergen-specific T_H2 cells, which in turn promote eosinophilia and further IgE production.

12-10 The clinical effects of allergic reactions vary according to the site of mast-cell activation.

When re-exposure to allergen triggers an allergic reaction, the effects are focused on the site at which mast-cell degranulation occurs. In the immediate response, the preformed mediators released are short-lived, and their potent effects on blood vessels and smooth muscles are therefore confined to the immediate vicinity of the activated mast cell. The more sustained effects of the late-phase response are also focused on the site of initial allergen-

Class of product	Examples	Biological effects
Enzyme	Tryptase, chymase, cathepsin G, carboxypeptidase	Remodeling of connective tissue matrix
Toxic mediator	Histamine, heparin	Toxic to parasites Increase vascular permeability Cause smooth muscle contraction
Cytokine	IL-4, IL-13	Stimulate and amplify T_H2 cell response
	IL-3, IL-5, GM-CSF	Promote eosinophil production and activation
	TNF-α (some stored preformed in granules)	Promotes inflammation, stimulates cytokine production by many cell types, activates endothelium
Chemokine	MIP-1α	Chemokinetic for monocytes, macrophages and neutrophils
Lipid mediator	Leukotrienes C4 and D4	Smooth muscle contraction Increased vascular permeability Mucus secretion
	Platelet-activating factor	Chemotactic to leukocytes Amplifies production of lipid mediators Neutrophil, eosinophil, and platelet activation

Fig. 12.11 Molecules synthesized and released by mast cells on stimulation by antigen binding to IgE. Mast cells release a wide variety of biologically active proteins and other chemical mediators. The lipid mediators derive from membrane phospholipids, which are cleaved to release the precursor molecule arachidonic acid. This molecule can be modified by two pathways to give rise to prostaglandins, thromboxanes, and leukotrienes. The leukotrienes, especially C4, D4 and E4, are important products of mast cells that sustain inflammatory responses in the tissues. Many anti-inflammatory drugs are inhibitors of arachidonic acid metabolism. Aspirin, for example, is an inhibitor of the enzyme cyclo-oxygenase and blocks the production of prostaglandins.

triggered activation, and the particular anatomy of this site may determine how readily the inflammation can be resolved. Thus, the clinical syndrome produced by an allergic reaction depends critically on three variables: the amount of allergen-specific IgE antibody present; the route by which the allergen is introduced; and the dose of allergen (Fig. 12.12).

12-11 The degranulation of mast cells in blood vessel walls after systemic absorption of allergen can cause generalized cardiovascular collapse.

If an allergen is given systemically or is rapidly absorbed from the gut, the connective tissue mast cells associated with all blood vessels can become activated. This activation causes a very dangerous syndrome called **systemic anaphylaxis**. Disseminated mast-cell activation causes a widespread increase in vascular permeability, leading to a catastrophic loss of blood pressure, constriction of the airways, and epiglottal swelling that can cause suffocation; this syndrome is called **anaphylactic shock**. This type of reaction can occur if drugs are administered to people with a specific allergy to that drug, or after an insect bite in individuals allergic to insect venom. Some foods, for example peanuts or brazil nuts, can be associated with systemic anaphylaxis. This syndrome can be rapidly fatal but can usually be controlled by the immediate injection of epinephrine (see Section 12-15).

The most frequent allergic reactions to drugs occur with penicillin and its relatives. In people with IgE antibodies against penicillin, administration of the drug by injection can cause anaphylaxis and even death. Great care should taken to avoid giving drugs to patients with a past history of allergy to the same drug or to one that is closely related structurally. Penicillin acts as a hapten (see Section 9-2); it is a small molecule with a highly reactive β-lactam

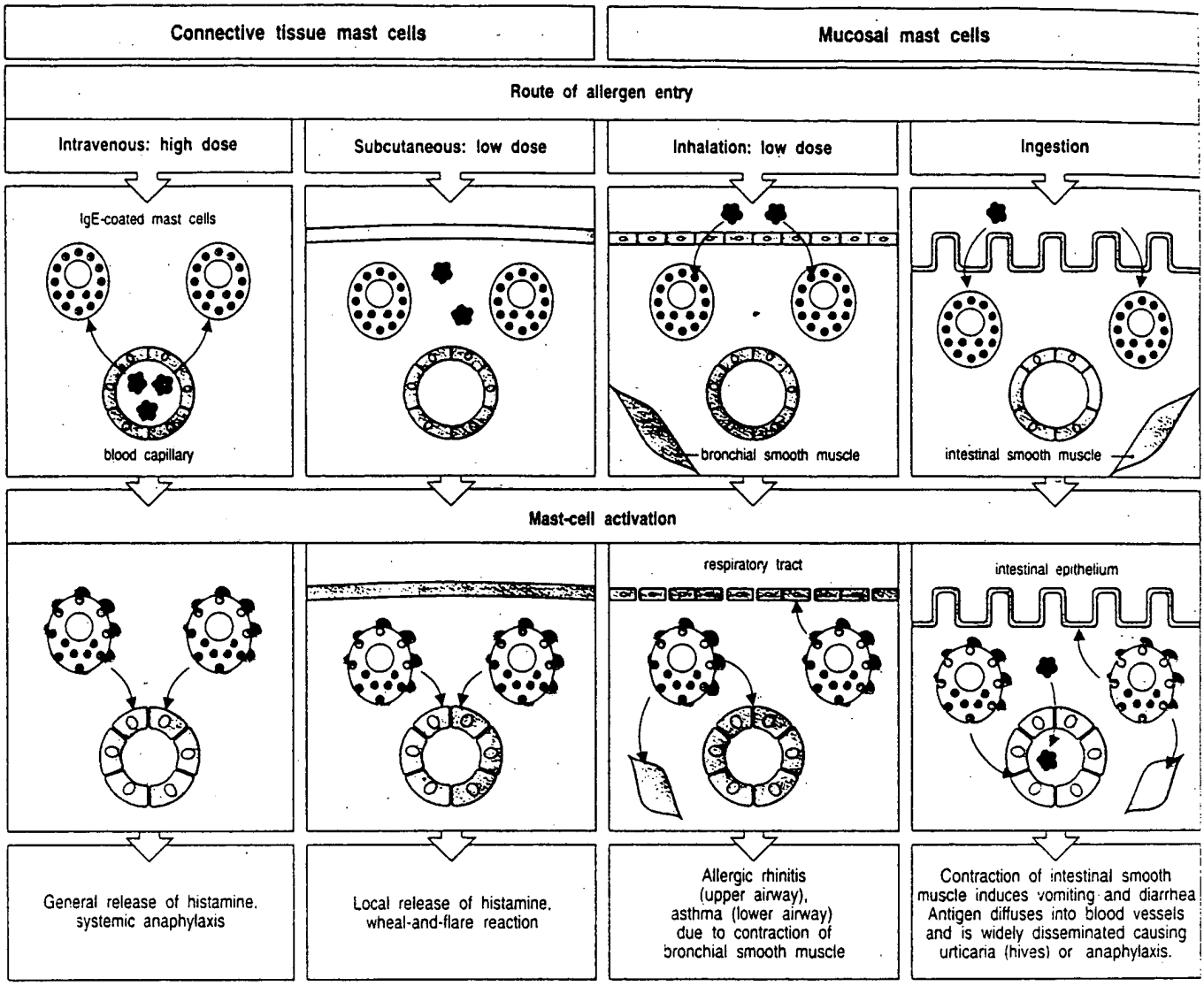

Fig. 12.12 The dose and route of allergen administration determine the type of IgE-mediated allergic reaction that results. There are two main anatomical distributions of mast cells: those associated with vascularized connective tissues, called connective tissue mast cells, and those found in submucosal layers of the gut and respiratory tract, called mucosal mast cells. In an allergic individual, all of these are loaded with IgE directed against specific allergens. The overall response to an allergen then depends on which mast cells are activated. Allergen in the bloodstream activates connective tissue mast cells throughout the body, resulting in the systemic release of histamine and other mediators. Subcutaneous administration of allergen activates only local connective tissue mast cells, leading to a local inflammatory reaction. Inhaled allergen, penetrating across epithelia, activates mainly mucosal mast cells, causing smooth muscle contraction in the lower airways; this leads to bronchoconstriction and difficulty in expelling inhaled air. Mucosal mast-cell activation also increases the local secretion of mucus by epithelial cells and causes irritation. Similarly, ingested allergen penetrates across gut epithelia, causing vomiting due to intestinal smooth muscle contraction; food allergens can also be disseminated in the bloodstream, causing urticaria (hives) when they reach the skin.

ring, crucial for its antibiotic activity. This ring reacts with amino groups on host proteins to form covalent conjugates. When penicillin is ingested or injected, it forms conjugates with self proteins, and these penicillin-modified self peptides can provoke a T_H2 response in some individuals. These T_H2 cells then activate penicillin-binding B cells to produce IgE antibody to the penicillin hapten. Thus, penicillin acts both as the B-cell antigen and, by modifying self peptides, as the T-cell antigen. When penicillin is injected intravenously into an allergic individual, the penicillin-modified proteins crosslink IgE molecules on the mast cells to cause anaphylaxis.

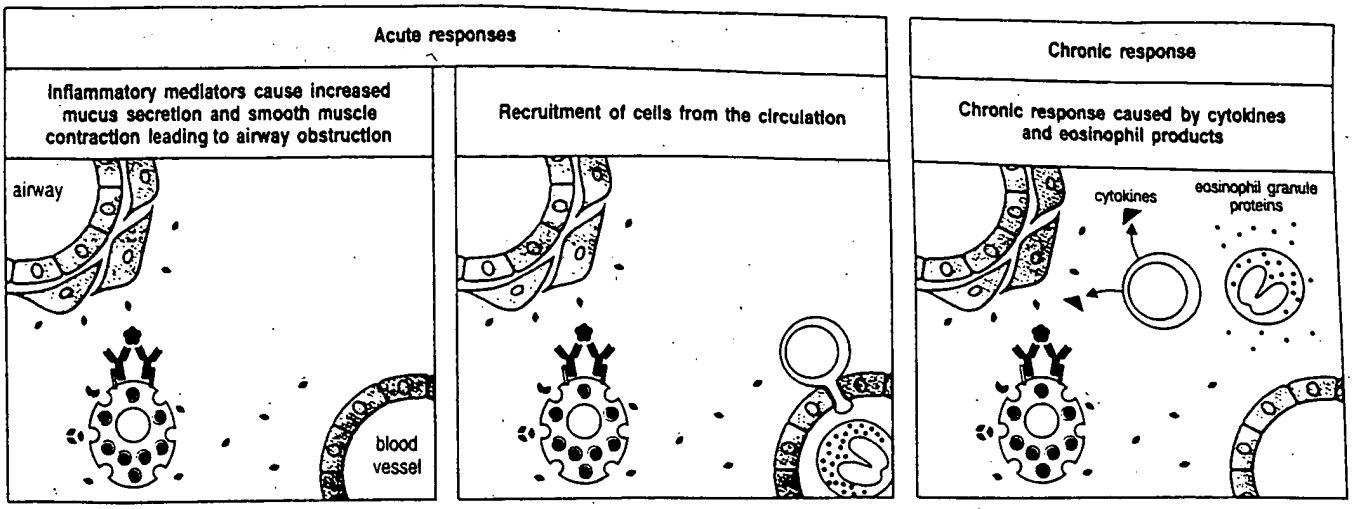

Fig. 12.13 The acute response in allergic asthma leads to T_H2-mediated chronic inflammation of the airways. In sensitized individuals, crosslinking of specific IgE on the surface of mast cells by inhaled allergen triggers them to secrete inflammatory mediators, causing bronchial smooth muscle contraction and an influx of inflammatory cells, including eosinophils, and T_H2 lymphocytes. Activated mast cells and T_H2 cells secrete cytokines that also augment eosinophil activation and degranulation, which causes further tissue injury and influx of inflammatory cells. The end result is chronic inflammation, which might then cause irreversible damage to the airways.

12-12 Allergen inhalation is associated with the development of rhinitis and asthma.

Inhalation is the most common route of allergen entry. Many people have mild allergies to inhaled antigens, manifesting as sneezing and a runny nose. This is called **allergic rhinitis**, and results from the activation of mucosal mast cells beneath the nasal epithelium by allergens that diffuse across the mucous membrane of the nasal passages. Allergic rhinitis is characterized by local edema leading to nasal obstruction, a nasal discharge, which is typically rich in eosinophils, and irritation of the nose from histamine release. A similar reaction to airborne allergens deposited on the conjunctiva of the eye is called allergic conjunctivitis. Allergic rhinitis and conjunctivitis are commonly caused by environmental allergens that are only present during certain seasons of the year. For example, hay fever is caused by a variety of allergens, including certain grass and tree pollens. Autumnal symptoms may be caused by weed pollen. These reactions are annoying but cause little lasting damage.

A more serious syndrome is **allergic asthma**, which is triggered by allergen-induced activation of submucosal mast cells in the lower airways (Fig. 12.13). This leads within seconds to bronchial constriction and increased secretion of fluid and mucus, making breathing more difficult by trapping inhaled air in the lungs. Patients with allergic asthma often need treatment, and asthmatic attacks can be life-threatening. An important feature of asthma is chronic inflammation of the airways, which is characterized by the continued presence of increased numbers of T_H2 lymphocytes, eosinophils, neutrophils, and other leukocytes (Fig. 12.14).

Although allergic asthma is initially driven by a response to a specific allergen, the subsequent chronic inflammation seems to be perpetuated even in the apparent absence of further exposure to allergen, and factors other than re-exposure to antigen can then trigger subsequent asthmatic

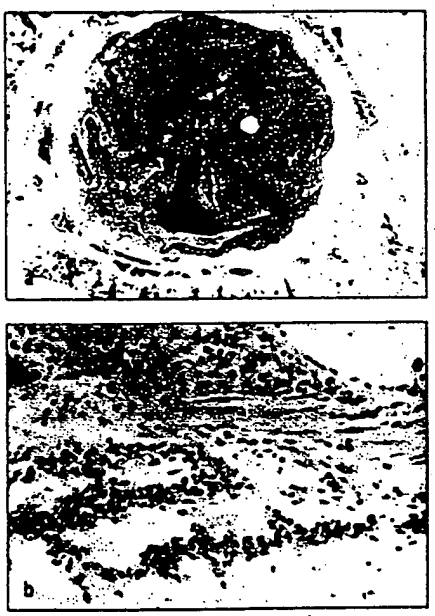

Fig. 12.14 Morphological evidence of chronic inflammation in the airways of an asthmatic patient. Panel a shows a section through a bronchus of a patient who died of asthma; there is almost total occlusion of the airway by a mucus plug. In panel b, a close-up view of the bronchial wall shows injury to the epithelium lining the bronchus, accompanied by a dense inflammatory infiltrate that includes eosinophils, neutrophils, and lymphocytes. Photographs courtesy of T Krausz.

attacks. For example, the airways of asthmatics characteristically show hyper-responsiveness to environmental chemical irritants such as cigarette smoke and sulfur dioxide. Bacterial, or more importantly viral, respiratory tract infections can exacerbate the disease by inducing a T_H2-dominated local response.

12-13 Skin allergy is manifest as urticaria or chronic eczema.

The same dichotomy between immediate and delayed responses is seen in cutaneous allergic responses. The skin forms an effective barrier to the entry of most allergens, but can be breached by local injection of small amounts of allergen, for example by a stinging insect. The entry of allergen causes a localized allergic reaction. Local mast-cell activation in the skin leads immediately to a local increase in vascular permeability, which causes extravasation of fluid. The mast-cell activation also stimulates a nerve axon reflex, causing the vasodilation of surrounding cutaneous blood vessels. The resulting skin lesion is called a **wheal-and-flare reaction**. About 8 hours later, a more widespread and sustained edematous response appears in some individuals as a consequence of the late-phase response (see Fig. 12.10). A disseminated form of the wheal-and-flare reaction, known as **urticaria** or hives, sometimes appears when ingested allergens enter the bloodstream and reach the skin. Histamine released by mast cells activated by allergen in the skin causes large, itchy red swellings beneath the skin.

Allergists take advantage of the immediate response to test for allergy by injecting minute amounts of potential allergens intracutaneously. Although the reaction after the administration of antigen by intracutaneous injection is usually very localized, there is a small risk of inducing systemic anaphylaxis. Another standard test for allergy is to measure levels of IgE antibody specific for a particular allergen in a sandwich ELISA (see Section 2-7).

Although acute urticaria is commonly caused by allergens, the causes of chronic urticaria, in which the urticarial rash can recur over long periods, are less well understood. In up to a third of cases, it seems likely that chronic urticaria is an autoimmune disease caused by autoantibodies against the α chain of $Fc\varepsilon RI$. This is an example of a type II hypersensitivity reaction (see Fig. 12.2) in which an autoantibody against a cellular receptor triggers cellular activation, in this case causing mast-cell degranulation with resulting urticaria.

A more prolonged inflammatory response is sometimes seen in the skin, most often in atopic children. They develop a persistent skin rash called **eczema**, due to a chronic inflammatory response similar to that seen in the bronchial walls of patients with asthma. The etiology of eczema is not well understood and it usually clears in adolescence, unlike rhinitis and asthma, which can persist throughout life.

12-14 Allergy to foods can cause symptoms limited to the gut but can also cause systemic reactions.

When an allergen is eaten, two types of allergic response are seen. Activation of mucosal mast cells associated with the gastrointestinal tract can lead to transepithelial fluid loss and smooth muscle contraction, generating vomiting and diarrhea. For reasons that are not understood, connective tissue mast cells in the deeper layers of the skin are also activated, presumably

by IgE antibodies binding to the ingested and absorbed allergen borne by the blood, resulting in urticaria. This is a common reaction when penicillin is ingested by a patient with penicillin-specific IgE antibodies.

Ingestion of food allergens can also lead to the development of generalized anaphylaxis, accompanied by cardiovascular collapse and acute asthmatic symptoms. Certain foods are particularly associated with this type of life-threatening response, an important one being peanuts.

12-15 Allergy can be treated by inhibiting either IgE production or the effector pathways activated by crosslinking of cell-surface IgE.

The approaches to the treatment and prevention of allergy are set out in Fig. 12.15. Two treatments are commonly used in clinical practice—desensitization and blockade of the effector pathways. There are also several approaches still in the experimental stage. In **desensitization**, the aim is to shift the antibody response away from an IgE-dominated response towards one dominated by IgG, which can prevent the allergen from activating IgE-mediated effector pathways. Patients are injected with escalating doses of allergen, starting with tiny amounts. This injection schedule seems gradually to divert the IgE-dominated response, driven by T_H2 cells, to one driven by T_H1 cells, with the consequent downregulation of IgE production. Recent evidence shows that desensitization is also associated with a reduction in the numbers of mast cells at the site of the allergic reaction. This procedure carries the risk of inducing IgE-mediated allergic responses as a complication of treatment.

An alternative and still experimental approach to desensitization is vaccination with peptides derived from common allergens. This procedure induces T-cell anergy *in vivo* (see Section 8-10) associated with multiple changes in the T-cell phenotype, including downregulation of cytokine production and of expression of the T-cell receptor:CD3 complex. IgE-mediated responses are not induced because IgE can recognize only the intact antigen. A major

Target step	Mechanism of treatment	Specific approach
T_H2 activation	Reverse T_H2/T_H1 balance	Injection of specific antigen or peptides Administration of cytokines e.g. IFN-γ, IL-10, IL-12, TGF-β
Activation of B cell to produce IgE	Block co-stimulation Inhibit T_H2 cytokines	Inhibit CD40L Inhibit IL-4 or IL-13
Mast-cell activation	Inhibit effects of IgE binding to mast cell	Blockade of IgE receptor
Mediator action	Inhibit effects of mediators on specific receptors Inhibit synthesis of specific mediators	Antihistamine drugs Lipo-oxygenase inhibitors
Eosinophil-dependent inflammation	Block cytokine and chemokine receptors that mediate eosinophil recruitment and activation	Inhibit IL-5 Block CCR3

Fig. 12.15 Approaches to the treatment of allergy. Possible methods of inhibiting allergic reactions are shown. Two approaches are in regular clinical use. The first is the injection of specific antigen in desensitization regimes, which are believed to divert the immune response to the allergen from a T_H2 to a T_H1 type, so that IgG is produced in place of IgE. The second clinically useful approach is the use of specific inhibitors to block the synthesis or effects of inflammatory mediators produced by mast cells.

difficulty with this approach is that individual responses to peptides are restricted by specific MHC class II alleles, and therefore different patients carrying different MHC class II alleles can respond to different allergen-derived peptides. As the human population is outbred, expressing a wide variety of MHC class II alleles, the number of peptides required to treat all allergic individuals might be very large.

The signaling pathways that enhance the IgE response in allergic disease are also potential targets for therapy. Inhibitors of IL-4, IL-5, and IL-13 would be predicted to reduce IgE responses, although redundancy between some of the activities of these cytokines might make this approach difficult to implement in practice. A second possible approach to manipulating the response is to give cytokines that promote T_H1-type responses. IFN-γ, IFN-α, IL-10, IL-12, and TGF-β have each been shown to reduce IL-4-stimulated IgE synthesis *in vitro*, and IFN-γ and IFN-α to reduce IgE synthesis *in vivo*.

Another target for therapeutic intervention might be the high-affinity IgE receptor. An effective competitor for IgE binding at this receptor could prevent the binding of antigen-specific IgE to the surfaces of mast cells, basophils, and eosinophils. Candidate competitor molecules include modified IgE Fc constructs that lack variable regions and are thus unable to bind antigen. Yet another approach would be to block the recruitment of eosinophils to sites of allergic inflammation. The eotaxin receptor CCR-3 is a potential target for this type of therapy. The production of eosinophils in bone marrow and their exit into the circulation might also be reduced by a blockade of IL-5 action.

The mainstays of therapy at present, however, are drugs that treat the symptoms of allergic disease and limit the inflammatory response that follows the activation of cells by the crosslinking of surface IgE by antigen. Anaphylactic reactions are treated with epinephrine, which stimulates the reformation of endothelial tight junctions, promotes the relaxation of constricted bronchial smooth muscle, and also stimulates the heart. Inhaled bronchodilators that act on β-adrenergic receptors to relax constricted muscle are also used to relieve acute asthma attacks. Antihistamines that block the histamine H1 receptor reduce the urticaria that follows histamine release from mast cells and eosinophils. Relevant H1 receptors include those on blood vessels that cause increased permeability to plasma and on unmyelinated nerve fibres that are thought to mediate the sensation of itch. In chronic allergic disease it is extremely important to treat and prevent the chronic inflammatory tissue injury. Topical or systemic corticosteroids (see Chapter 14) are used to suppress the chronic inflammatory changes seen in asthma, rhinitis, and eczema.

Summary.

The allergic response to innocuous antigens reflects the pathophysiological aspect of a defensive response with the physiological role of protecting hosts against helminthic parasites. It is triggered by IgE antibodies bound to the high-affinity IgE receptor FcεRI on mast cells. Mast cells are strategically distributed beneath the mucosal surfaces of the body and in connective tissue. The resulting inflammation can be divided into early events, characterized by rapidly dispersed mediators such as histamine, and later events that involve leukotrienes, cytokines, and chemokines, which recruit and activate eosinophils in particular, but also basophils. The late phase of this response can evolve into chronic inflammation, characterized by the presence of effector T cells and eosinophils, which is most clearly seen in allergic asthma.

Hypersensitivity diseases.

In the first part of this chapter we saw how IgE is involved in allergic disease, also known as type I hypersensitivity. Immunological responses involving IgG antibodies or specific T cells can also cause adverse hypersensitivity reactions. Although these effector arms of the immune response normally participate in protective immunity to infection, they occasionally react with non-infectious antigens to produce acute or chronic hypersensitivity reactions. We shall describe common examples of such reactions in this last part of the chapter.

12-16 Innocuous antigens can cause type II hypersensitivity reactions in susceptible individuals by binding to the surfaces of circulating blood cells.

Antibody-mediated destruction of red blood cells (hemolytic anemia) or platelets (thrombocytopenia) is an uncommon side-effect associated with the intake of certain drugs such as the antibiotic penicillin, the anti-cardiac arrhythmia drug quinidine, or the anti-hypertensive agent methyldopa. These are examples of **type II hypersensitivity reactions** in which the drug binds to the cell surface and serves as a target for anti-drug IgG antibodies (see Fig. 12.2). The anti-drug antibodies are made in only a minority of individuals and it is not clear why these individuals are susceptible to developing them. The cell-bound antibody triggers clearance of the cell from the circulation, predominantly by tissue macrophages in the spleen, which bear Fcγ receptors.

12-17 Systemic disease caused by immune complex formation can follow the administration of large quantities of poorly catabolized antigens.

Type III hypersensitivity reactions can arise when the antigen is soluble. The pathology is caused by the deposition of antigen:antibody aggregates or **immune complexes** in certain tissue sites. Immune complexes are generated in all antibody responses but their pathogenic potential is determined, in part, by their size. Larger aggregates fix complement and are readily cleared from the circulation by the mononuclear phagocytic system. The small complexes that form at antigen excess, however, tend to deposit in blood vessel walls. There they can ligate Fc receptors on leukocytes, leading to leukocyte activation and tissue injury.

A local type III hypersensitivity reaction can be triggered in the skin of sensitized individuals possessing IgG antibodies against the sensitizing antigen. When antigen is injected into the skin, IgG antibody that has diffused into the tissues forms immune complexes locally. The immune complexes bind Fc receptors on mast cells and other leukocytes, which creates a local inflammatory response with increased vascular permeability. The enhanced vascular permeability allows fluid and cells, especially polymorphonuclear leukocytes, to enter the site from the local vessels. This reaction is called an **Arthus reaction** (Fig. 12.16). The immune complexes also activate complement, releasing C5a, which contributes to the inflammatory reaction. The

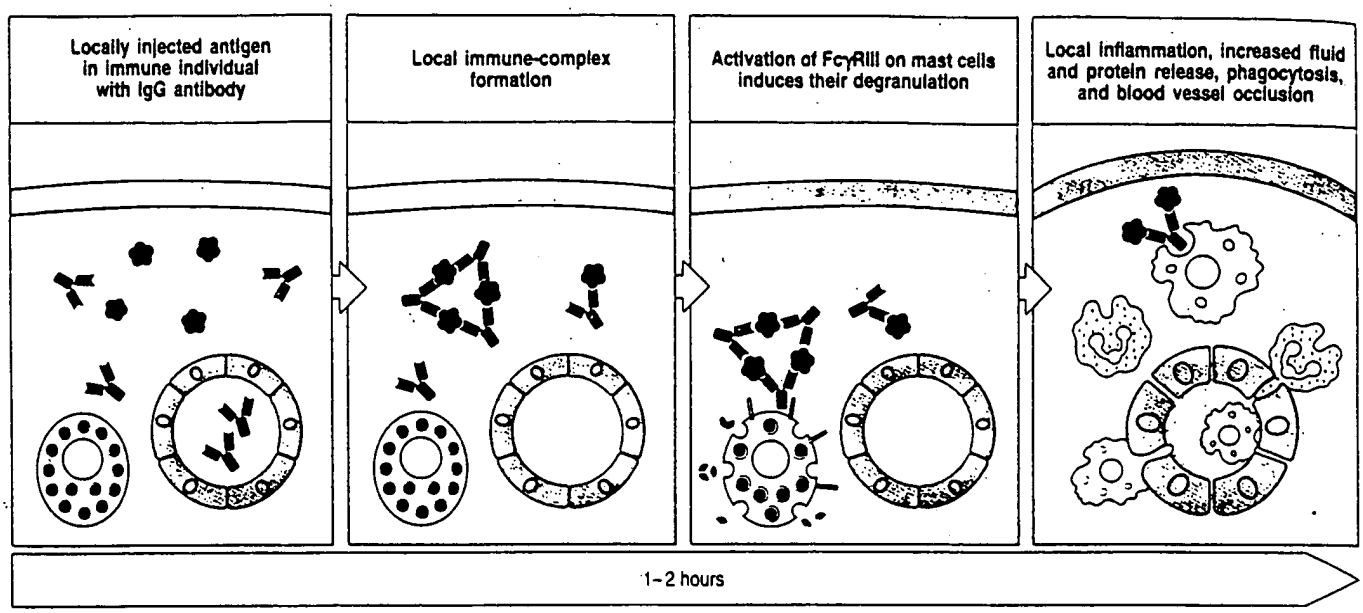

Fig. 12.16 The deposition of immune complexes in local tissues causes a local inflammatory response known as an Arthus reaction (type III hypersensitivity reaction). In individuals who have already made IgG antibody against an antigen, the same antigen injected into the skin forms immune complexes with IgG antibody that has diffused out of the capillaries. Because the dose of antigen is low, the immune complexes are only formed close to the site of injection, where they activate Fcγ receptor-bearing mast cells. As a result of mast-cell activation, inflammatory cells invade the site, and blood vessel permeability and blood flow are increased. Platelets also accumulate inside the vessel at the site, ultimately leading to vessel occlusion.

Arthus reaction is absent in mice lacking expression of the α or γ chain of the FcγRIII receptor (CD16) on mast cells, but remains largely unperturbed in complement-deficient mice, showing the primary importance of FcγRIII in triggering inflammatory responses.

A systemic type III hypersensitivity reaction, known as **serum sickness**, can result from the injection of large quantities of a poorly catabolized foreign antigen. This illness was so named because it frequently followed the administration of therapeutic horse antiserum. In the pre-antibiotic era, antiserum made by immunizing horses was often used to treat pneumococcal pneumonia; the specific anti-pneumococcal antibodies in the horse serum would help the patient to clear the infection. In much the same way, antivenin (serum from horses immunized with snake venoms) is still used today as a source of neutralizing antibodies to treat people suffering from the bites of poisonous snakes.

Serum sickness occurs 7–10 days after the injection of the horse serum, a time interval that corresponds to the time for a primary immune response to be developed against the foreign antigen. The clinical features of serum sickness are chills, fevers, rash, arthritis, and sometimes glomerulonephritis. Urticaria is a prominent feature of the rash, implying a role for histamine derived from mast-cell degranulation. In this case the mast-cell degranulation is triggered by the ligation of cell-surface FcγRIII by immune complexes.

The course of serum sickness is illustrated in Fig. 12.17. The onset of disease coincides with the development of antibodies against the abundant soluble proteins in horse serum; these antibodies form immune complexes with their antigens throughout the body. These immune complexes fix complement and can bind to and activate leukocytes bearing Fc and complement receptors; these in turn cause widespread tissue injury. The formation of immune

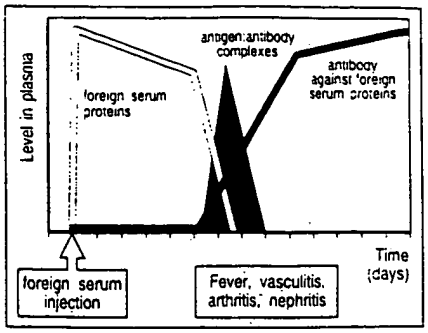

Fig. 12.17 Serum sickness is a classic example of a transient immune-compl x-mediated syndr me. An injection of a foreign protein or proteins, in this case those of horse serum, leads to an antibody response. These antibodies form immune complexes with the circulating foreign proteins. The complexes are deposited in small vessels and activate complement and phagocytes, inducing fever and the symptoms of vasculitis, nephritis, and arthritis. All these effects are transient and resolve when the foreign protein is cleared.

complexes causes clearance of the foreign antigen and so serum sickness is usually a self-limiting disease. Serum sickness after a second dose of horse antiserum follows the kinetics of a secondary antibody response and the onset of disease occurs typically within a day or two. Serum sickness is nowadays seen after the use of anti-lymphocyte globulin, which is used as an immunosuppressive agent in transplant recipients (see Chapter 14), and also rarely after the administration of streptokinase, a bacterial enzyme that is used as a thrombolytic agent to treat patients with a myocardial infarction or heart attack.

A similar type of immunopathological response is seen in two other situations in which antigen persists. The first is when an adaptive antibody response fails to clear an infectious agent, for example in subacute bacterial endocarditis or in chronic viral hepatitis. In this situation, the multiplying bacteria or viruses are continuously generating new antigen in the presence of a persistent antibody response, which fails to eliminate the organism. Immune complex disease ensues, with injury to small blood vessels in many tissues and organs including the skin, kidneys, and nerves. Immune complexes also form in autoimmune diseases such as systemic lupus erythematosus where, because the antigen persists, the deposition of immune complexes continues, and serious disease can result (see Section 13-7).

Some inhaled allergens provoke IgG rather than IgE antibody responses, perhaps because they are present at relatively high levels in inhaled air. When a person is re-exposed to high doses of such inhaled antigens, immune complexes form in the alveolar wall of the lung. This leads to the accumulation of fluid, protein, and cells in the alveolar wall, slowing blood–gas interchange and compromising lung function. This type of reaction occurs in certain occupations such as farming, where there is repeated exposure to hay dust or mold spores. The disease that results is therefore called **farmer's lung**. If exposure to antigen is sustained, the alveolar membranes can become permanently damaged.

12-18 Delayed-type hypersensitivity reactions are mediated by T_H1 cells and CD8 cytotoxic T cells.

Unlike the immediate hypersensitivity reactions described so far, which are mediated by antibodies, **delayed-type hypersensitivity** or **type IV hypersensitivity reactions** are mediated by specific T cells. Such effector T cells function in essentially the same way as during a response to an infectious pathogen, as described in Chapter 8. The causes and consequences of some syndromes in which type IV hypersensitivity responses predominate are listed in Fig. 12.18. These responses are clearly caused by T cells, because they can be seen in individuals who lack immunoglobulin. Such responses can also be transferred between experimental animals by using pure T cells or cloned T-cell lines.

Fig. 12.18 Type IV hypersensitivity responses. These reactions are mediated by T cells and all take some time to develop. They can be grouped into three syndromes, according to the route by which antigen passes into the body. In delayed-type hypersensitivity the antigen is injected into the skin; in contact hypersensitivity it is absorbed into the skin; and in gluten-sensitive enteropathy it is absorbed by the gut.

Type IV hypersensitivity reactions are mediated by antigen-specific effector T cells

Syndrome	Antigen	Consequence
Delayed-type hypersensitivity	Proteins: Insect venom Mycobacterial proteins (tuberculin, lepromin)	Local skin swelling: Erythema Induration Cellular infiltrate Dermatitis
Contact hypersensitivity	Haptens: Pentadecacatechol (poison ivy) DNFB Small metal ions: Nickel Chromate	Local epidermal reaction: Erythema Cellular infiltrate Contact dermatitis
Gluten-sensitive enteropathy (celiac disease)	Gliadin	Villous atrophy in small bowel Malabsorption

The prototypic delayed-type hypersensitivity reaction is an artifact of modern medicine—the tuberculin test (see Section 2-21). This is used to determine whether an individual has previously been infected with *Mycobacterium tuberculosis*. When small amounts of a protein from *M. tuberculosis* are injected subcutaneously, a T-cell mediated local inflammatory reaction evolves over 24–72 hours in individuals who have previously responded to this pathogen. The response is mediated by T_H1 cells, which enter the site of antigen injection, recognize complexes of peptide:MHC class II on antigen-presenting cells, and release inflammatory cytokines that increase local blood vessel permeability, bringing plasma into the tissue and recruiting accessory cells to the site; this causes a visible swelling (Fig. 12.19). Each of these phases takes several hours and so the mature response appears only 24–48 hours after challenge. The cytokines produced by the activated T_H1 cells and their actions are shown in Fig. 12.20.

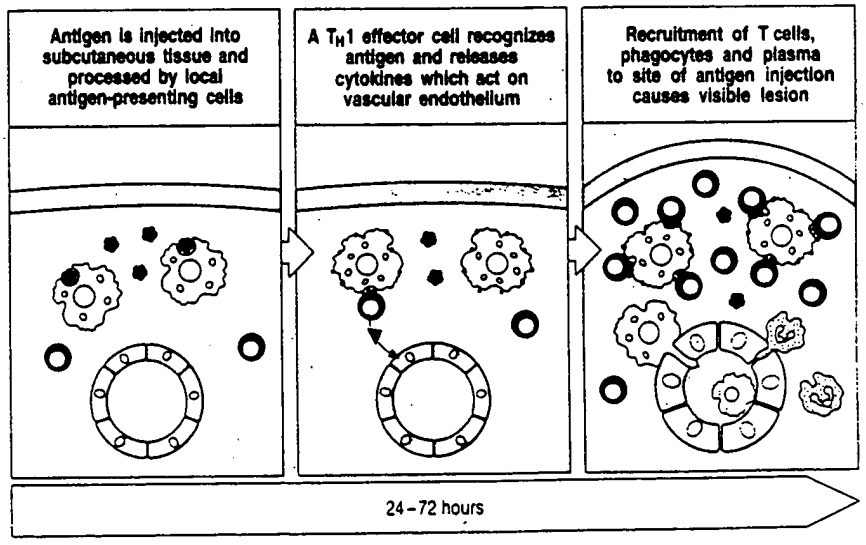

Fig. 12.19 The stages of a delayed-type hypersensitivity reaction. The first phase involves uptake, processing, and presentation of the antigen by local antigen-presenting cells. In the second phase, T_H1 cells that were primed by a previous exposure to the antigen migrate into the site of injection and become activated. Because these specific cells are rare, and because there is no inflammation to attract cells into the site, it can take several hours for a T cell of the correct specificity to arrive. These cells release mediators that activate local endothelial cells, recruiting an inflammatory cell infiltrate dominated by macrophages and causing the accumulation of fluid and protein. At this point, the lesion becomes apparent.

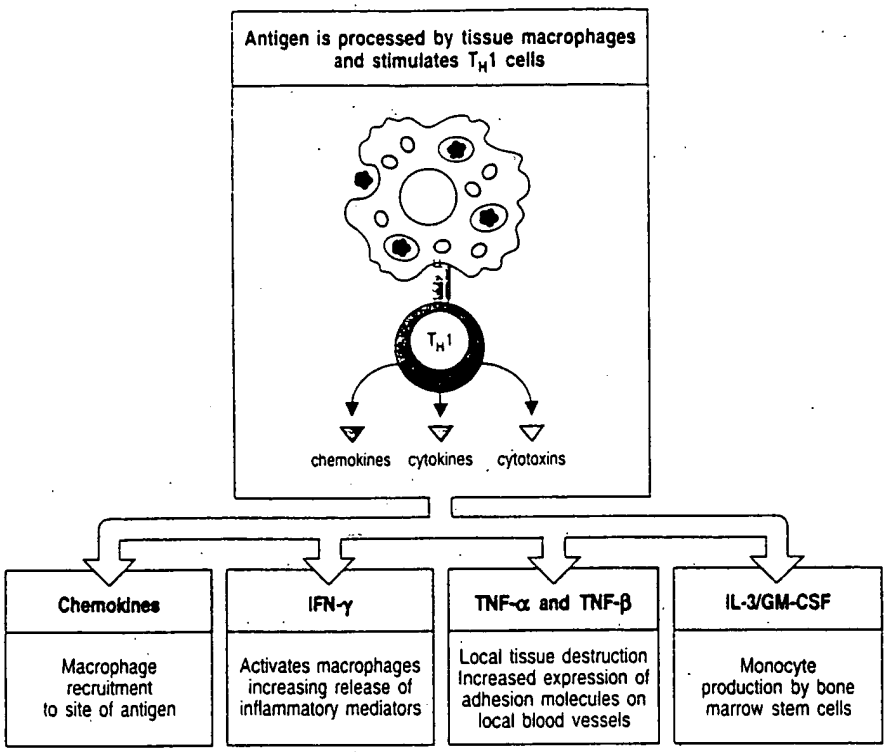

Fig. 12.20 The delayed-type (type IV) hypersensitivity resp ns is dir cted by cyt kines released by T_H1 cells stimulated by antigen. Antigen in the local tissues is processed by antigen-presenting cells and presented on MHC class II molecules. Antigen-specific T_H1 cells that recognize the antigen locally at the site of injection release chemokines and cytokines that recruit macrophages to the site of antigen deposition. Antigen presentation by the newly recruited macrophages then amplifies the response. T cells can also affect local blood vessels through the release of TNF-α and the cytotoxin TNF-β, and stimulate the production of macrophages through the release of IL-3 and GM-CSF. Finally, T_H1 cells activate macrophages through the release of IFN-γ and TNF-α, and kill macrophages and other sensitive cells through TNF-β or by the cell-surface expression of the Fas ligand.

Very similar reactions are observed in several cutaneous hypersensitivity responses. For instance, the rash produced by poison ivy (Fig. 12.21) is caused by a T-cell response to a chemical in the poison ivy leaf called pentadecacatechol. This compound binds covalently to host proteins. The modified self proteins are then cleaved into modified self peptides, which can bind to self MHC class II molecules and be recognized by T_H1 cells. When specifically sensitized T cells recognize these complexes, they can produce extensive inflammation (Fig. 12.22). As the chemical is delivered by contact with the skin, this is called a **contact hypersensitivity reaction**. The compounds that cause such reactions must be chemically active so that they can form stable complexes with host proteins.

Some insect proteins also elicit delayed-type hypersensitivity responses. However, the early phases of the host reaction to an insect bite are often IgE-mediated or the result of the direct effects of insect venoms. Finally, some unusual delayed-type hypersensitivity responses to divalent cations have been observed, for example to nickel, which can alter the conformation or peptide binding of MHC class II molecules, and thus provoke a T-cell response.

Type IV hypersensitivity reactions can also involve CD8 T cells, which damage tissues mainly by cell-mediated cytotoxicity. Some chemicals, including pentadecacatechol, are soluble in lipid and can therefore cross the cell membrane and modify intracellular proteins. These modified proteins generate modified peptides within the cytosol, which are translocated into the endoplasmic reticulum and are delivered to the cell surface by MHC class I molecules. These are recognized by CD8 T cells, which can cause damage either by killing the eliciting cell or by secreting cytokines such as IFN-γ.

Fig. 12.21 Blistering skin lesi ns on hand of patient with poison ivy contact d rmatitis. Photograph courtesy of R Geha.

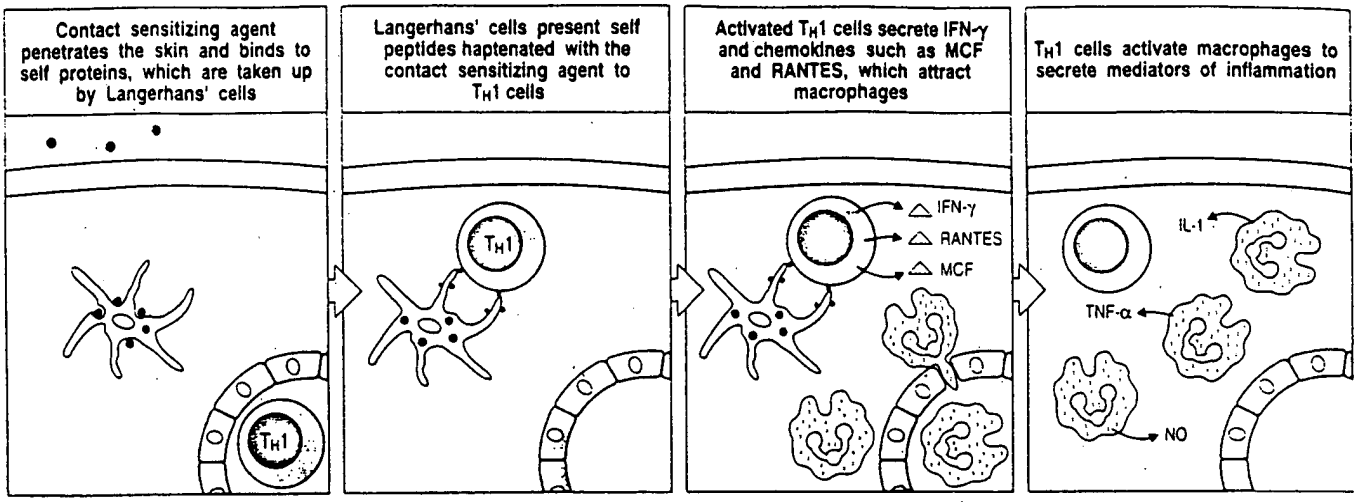

Fig. 12.22 Development of a delayed-type hypersensitivity response to a contact-sensitizing agent such as the pentadecacatechol in poison ivy. The contact-sensitizing agent is a small lipid-soluble molecule that can easily penetrate intact skin. It binds covalently as a hapten to a variety of endogenous proteins, which are taken up and processed by Langerhans' cells, the major antigen-presenting cells of skin. These present haptenated peptides to effector T_H1 cells (which must have been previously primed in lymph nodes and then have traveled back to the skin). These then secrete cytokines and chemokines, which in turn attract monocytes and induce their maturation into activated tissue macrophages, which contribute to the inflammatory lesions depicted in Fig. 12.21.

Summary.

Hypersensitivity diseases reflect normal immune mechanisms directed against innocuous antigens. They can be mediated by IgG antibodies bound to modified cell surfaces, or by complexes of antibodies bound to poorly catabolized antigens, as occurs in serum sickness. Hypersensitivity reactions mediated by T cells can be activated by modified self proteins, or by injected proteins such as the mycobacterial extract tuberculin. These T-cell mediated responses require the induced synthesis of effector molecules and develop more slowly, which is why they are termed delayed-type hypersensitivity.

Summary to Chapter 12.

Immune responses to otherwise innocuous antigens produce allergic or hypersensitive reactions upon re-exposure to the same antigen. Most allergies involve the production of IgE antibody to common environmental allergens. Some people are intrinsically prone to making IgE antibodies against many allergens, and such people are said to be atopic. IgE production is driven by antigen-specific T_H2 cells, which are initially primed in the presence of a burst of IL-4 released by specialized T cells early in the immune response. The IgE produced binds to the high-affinity IgE receptor FcεRI on mast cells, basophils, and activated eosinophils. The physiological role of this system is to provide front-line defense against pathogens but, in economically developed societies, it is more frequently involved in allergic reactions. Eosinophils and specific effector T cells have an extremely important role in chronic allergic inflammation, which is the major cause of the chronic morbidity of asthma. Antibodies of other isotypes and specific effector T cells contribute to hypersensitivity to other antigens.

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